MANAGEMENT OF OPHTHALMOLOGIC DISORDERS, INCLUDING MACULAR DEGENERATION

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No. 60/545,456, filed February 17, 2004; U.S. Provisional Patent Application Ser. No. 60/567,604, filed May 3, 2004; and U.S. Provisional Patent Application Ser. No. 60/578,324, filed June 9, 2004, all of which are hereby incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Support for research leading to subject matter of this application was provided in part by the National Institutes of Health Grant No. R01-EY-04096. Accordingly, the United States Government has certain rights with respect to subject matter of this application.

INTRODUCTION

[0003] Age related diseases of vision are an ever-increasing health problem in industrial societies. Age related macular degeneration (AMD) affects millions of persons worldwide and is a leading cause of vision loss and blindness in ageing populations. In this disease, daytime vision (cone dominated vision) degrades with time because cone photoreceptors, which are concentrated in the foveal region of the retina, die. The incidence of this disease increases from less than 10% of the population 50 years of age to over 30% at 75 and continues upwards past this age. The onset of the disease has been correlated with the accumulation of complex and toxic biochemicals in and around the retinal pigment epithelium (RPE) and lipofuscin in the RPE. The accumulation of these retinotoxic mixtures is one of the most important known risk factors in the etiology of AMD.

[0004] The RPE forms part of the retinal-blood barrier and also supports the function of photoreceptor cells, including rods and cones. Among other activities, the RPE routinely phagocytoses spent outer segments of rod cells. In at least some forms of macular degeneration, accumulation of lipofuscin in the RPE is due in part to this phagocytosis. Retinotoxic compounds form in the discs of rod photoreceptor outer segments. Consequently, the retinotoxic compounds in the disc are brought into the RPE, where they impair further phagocytosis of outer segments and cause apoptosis of the RPE.

Photoreceptors cells, including cone cells essential for daytime vision, then die, denuded of RPE support.

[0005] One of the retinotoxic compounds formed in the discs of rod outer segments is *N*-retinylidene-*N*-retinylethanolamine (A_2E), which is an important component of the retinotoxic lipofuscins. A_2E is normally formed in the discs but in such small amounts that it does not impair RPE function upon phagocytosis. However, in certain pathological conditions, so much A_2E can accumulate in the disc that the RPE is "poisoned" when the outer segment is phagocytosed.

[0006] A_2E is produced from all-*trans*-retinal, one of the intermediates of the rod cell visual cycle. During the normal visual cycle (summarized in Figure 1), all-*trans*-retinal is produced inside rod outer-segment discs. The all-*trans*-retinal can react with phosphatidylethanolamine (PE), a component of the disc membrane, to form *N*-retinylidene-PE. Rim protein (RmP), an ATP-binding cassette transporter located in the membranes of rod outer-segment discs, then transports all-*trans*-retinal and/or *N*-retinylidene-PE out of the disc and into rod outer-segment cytoplasm. The environment there favors hydrolysis of the *N*-retinylidene-PE. The all-*trans*-retinal is reduced to all-*trans*-retinol in the rod cytoplasm. The all-*trans*-retinol then crosses the rod outer-segment plasma membrane into the extracellular space and is taken up by cells of the retinal pigment epithelium (RPE). The all-*trans*-retinol is converted through a series of reactions to 11-*cis*-retinal, which returns to the photoreceptor and continues in the visual cycle.

[0007] However, defects in RmP can derange this process by impeding removal of all-*trans*-retinal from the disc. In a recessive form of macular degeneration called Stargardt's disease (1/10,000 incidence rate often affecting children; 25,00 affected individuals in the U.S.), the gene encoding RmP, *abcr,* is mutated, and the transporter is nonfunctional. As a result, all-*trans*-retinal and/or *N*-retinylidene-PE become trapped in the disc. The *N*-retinylidene-PE can then react with another molecule of all-*trans*-retinal to form *N*-retinylidene-*N*-retinylethanolamine (A_2E); this is summarized in Figure 2. As noted above, some A_2E is formed even under normal conditions; however, its production is greatly increased when its precursors accumulate inside the discs due to the defective transporter, and can thereby cause macular degeneration.

[0008] Other forms of macular degeneration may also result from pathologies that result in lipofuscin accumulation. A dominant form of Stargardt's disease, known as chromosome

6-linked autosomal dominant macular dystrophy (ADMD, OMIM #600110), is caused by a mutation in the gene encoding elongation of very long chain fatty acids-4, *elovl4*.

[0009] There are few, if any, preventative treatments for AMD, and therapeutic interventions are available for only certain, less common, forms of the disease.

SUMMARY

[0010] This disclosure relates to compositions, systems, and methods for managing macular degeneration, and, more specifically, for preventing the accumulation of retinotoxic compounds in and around the retinal pigment epithelium.

[0011] In one embodiment, the accumulation of A_2E in rod outer-segment discs is prevented or reduced. It has been found that A_2E production in discs can be reduced by administering a drug that limits the visual cycle. The limitation can be achieved in a number of ways. In one approach, a drug can effectively short-circuit the portion of the visual cycle that generates the A_2E precursor, all-*trans*-retinal. In another approach, a drug can inhibit particular steps in the visual cycle necessary for synthesizing all-*trans*-retinal. In yet another approach, a drug can prevent binding of intermediate products (retinyl esters) to certain chaperone proteins in the retinal pigment epithelium.

[0012] In one embodiment, a method of treating or preventing macular degeneration in a subject may include administering to the subject a drug that short-circuits the visual cycle at a step of the visual cycle that occurs outside a disc of a rod photoreceptor cell. In another embodiment, a method of treating or preventing macular degeneration in a subject may include administering to the subject a drug that inhibits and/or interferes with at least one of lecithin retinol acyl transferase, RPE65, 11-*cis*-retinol dehydrogenase, and isomerohydrolase.

[0013] In yet another embodiment, a method of identifying a macular degeneration drug may include administering a candidate drug to a subject having, or at risk for developing, macular degeneration, and measuring accumulation of a retinotoxic compound in the retinal pigment epithelium of the subject.

[0014] A wide variety of drugs are contemplated for use. In some embodiments, inhibitors of the visual cycle include retinoic acid analogs. In other embodiments, drugs that short circuit the visual cycle include aromatic amines and hydrazines.

BRIEF DESCRIPTION OF THE FIGURES

[0015] Figure 1 depicts the visual cycle.

[0016] Figure 2 depicts the synthesis of A_2E.

[0017] Figure 3 depicts an intervention for short-circuiting the visual cycle.

[0018] Figures 4A-C depicts data concerning the binding of all-*trans*-retinoic acid to RPE65.

[0019] Figures 5A-C depicts data concerning the binding of 13-*cis*-retinoic acid to RPE65.

[0020] Figures 6A-C depicts data concerning the binding of N-(4-hydroxyphenyl)retinamide (4-HPR) to RPE65.

[0021] Figure 7 depicts data concerning competitive binding between all-*trans*-retinoic acid and all-*trans*-retinyl palmitate to RPE65.

[0022] Figure 8 depicts data concerning the effect of all-*trans* Retinoic acid (atRA), 13-*cis*-Retinoic acid (13cRA) and N-(4-hydroxyphenyl)retinamide (4-HPR) on 11-*cis*-retinol biosynthesis.

[0023] Figures 9 A1, A2, B1, and B2 depict data concerning the binding of all-*trans*-retinol and all-*trans*-retinyl palmitate to purified sRPE65. Figure 9C depicts data concerning binding of vitamin A to sRPE65. Figure 9D lists binding constants measured for various binding partners.

[0024] Figures 10A-C depict data concerning *in vivo* palmitoylation of mRPE65.

[0025] Figures 11 A-D depict data concerning interconversion of mRPE65 and sRPE65.

[0026] Figures 12 A-C depict data concerning palmitoylation of 11-*cis*-retinol.

[0027] Figures 13 A and B depict how regulatory elements described might direct the flow of retinoids in vision.

[0028] Figures 14A-18B present data regarding in vivo effects of short circuit drugs.

[0029] Figures 19-24 present data regarding in vivo effects of enzyme inhibitors and/or RPE65 antagonists.

[0030] Figure 25 presents data concerning in vitro formation of A_2E in the presence of aromatic amines.

DETAILED DESCRIPTION

[0031] 1. Overview

[0032] The present disclosure provides compositions and methods for managing macular degeneration by preventing or reducing the accumulation of A_2E in rod outer-segment discs. A_2E accumulation can be prevented or reduced by decreasing the amount of all-*trans*-retinal present in discs of rod outer segments. In one approach, a drug may be administered that inhibits one or more enzymatic steps in the visual cycle, so that production of all-*trans*-retinal is diminished. In another approach, a drug may be

administered that drives the isomerization of 11-*cis*-retinal to all-*trans*-retinal in the RPE, thereby decreasing the amount 11-*cis*-retinal that returns to the outer segment discs to be re-isomerized to all-*trans*-retinal.

[0033] 2. Definitions

[0034] For convenience, before further description of exemplary embodiments, certain terms employed in the specification, examples, and appended claims are collected here. These definitions should be read in light of the remainder of the disclosure and as understood by a person of skill in the art.

[0035] The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0036] The term "access device" is an art-recognized term and includes any medical device adapted for gaining or maintaining access to an anatomic area. Such devices are familiar to artisans in the medical and surgical fields. An access device may be a needle, a catheter, a cannula, a trocar, a tubing, a shunt, a drain, or an endoscope such as an otoscope, nasopharyngoscope, bronchoscope, or any other endoscope adapted for use in the joint area, or any other medical device suitable for entering or remaining positioned within the preselected anatomic area.

[0037] The terms "biocompatible compound" and "biocompatibility" when used in relation to compounds are art-recognized. For example, biocompatible compounds include compounds that are neither themselves toxic to the host (e.g., an animal or human), nor degrade (if the compound degrades) at a rate that produces monomeric or oligomeric subunits or other byproducts at toxic concentrations in the host. In certain embodiments, biodegradation generally involves degradation of the compound in an organism, e.g., into its monomeric subunits, which may be known to be effectively non-toxic. Intermediate oligomeric products resulting from such degradation may have different toxicological properties, however, or biodegradation may involve oxidation or other biochemical reactions that generate molecules other than monomeric subunits of the compound. Consequently, in certain embodiments, toxicology of a biodegradable compound intended for *in vivo* use, such as implantation or injection into a patient, may be determined after one or more toxicity analyses. It is not necessary that any subject composition have a purity of 100% to be deemed biocompatible; indeed, it is only necessary that the subject compositions be biocompatible as set forth above. Hence, a subject composition may

comprise compounds comprising 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75% or even less of biocompatible compounds, e.g., including compounds and other materials and excipients described herein, and still be biocompatible.

[0038] To determine whether a compound or other material is biocompatible, it may be necessary to conduct a toxicity analysis. Such assays are well known in the art. One example of such an assay may be performed with live carcinoma cells, such as GT3TKB tumor cells, in the following manner: the sample is degraded in 1M NaOH at 37 °C until complete degradation is observed. The solution is then neutralized with 1M HCl. About 200 μL of various concentrations of the degraded sample products are placed in 96-well tissue culture plates and seeded with human gastric carcinoma cells (GT3TKB) at 10^4/well density. The degraded sample products are incubated with the GT3TKB cells for 48 hours. The results of the assay may be plotted as % relative growth vs. concentration of degraded sample in the tissue-culture well. In addition, compounds and formulations may also be evaluated by well-known *in vivo* tests, such as subcutaneous implantations in rats to confirm that they do not cause significant levels of irritation or inflammation at the subcutaneous implantation sites.

[0039] The term "biodegradable" is art-recognized, and includes compounds, compositions and formulations, such as those described herein, that are intended to degrade during use. Biodegradable compounds typically differ from non-biodegradable compounds in that the former may be degraded during use. In certain embodiments, such use involves *in vivo* use, such as *in vivo* therapy, and in other certain embodiments, such use involves *in vitro* use. In general, degradation attributable to biodegradability involves the degradation of a biodegradable compound into its component subunits, or digestion, e.g., by a biochemical process, of the compound into smaller subunits. In certain embodiments, two different types of biodegradation may generally be identified. For example, one type of biodegradation may involve cleavage of bonds (whether covalent or otherwise) in the compound. In such biodegradation, monomers and oligomers typically result, and even more typically, such biodegradation occurs by cleavage of a bond connecting one or more of substituents of a compound. In contrast, another type of biodegradation may involve cleavage of a bond (whether covalent or otherwise) internal to side chain or that connects a side chain to the compound. For example, a therapeutic agent or other chemical moiety attached as a side chain to the compound may be released by biodegradation. In certain embodiments, one or the other or both generally types of biodegradation may occur during use of a compound.

As used herein, the term "biodegradation" encompasses both general types of biodegradation.

[0040] The degradation rate of a biodegradable compound often depends in part on a variety of factors, including the chemical identity of the linkage responsible for any degradation, the molecular weight, crystallinity, biostability, and degree of cross-linking of such compound, the physical characteristics of the implant, shape and size, and the mode and location of administration. For example, the greater the molecular weight, the higher the degree of crystallinity, and/or the greater the biostability, the biodegradation of any biodegradable compound is usually slower. The term "biodegradable" is intended to cover materials and processes also termed "bioerodible".

[0041] In certain embodiments, if the biodegradable compound also has a therapeutic agent or other material associated with it, the biodegradation rate of such compound may be characterized by a release rate of such materials. In such circumstances, the biodegradation rate may depend on not only the chemical identity and physical characteristics of the compound, but also on the identity of any such material incorporated therein.

[0042] In certain embodiments, compound formulations biodegrade within a period that is acceptable in the desired application. In certain embodiments, such as *in vivo* therapy, such degradation occurs in a period usually less than about five years, one year, six months, three months, one month, fifteen days, five days, three days, or even one day on exposure to a physiological solution with a pH between 6 and 8 having a temperature of between 25 and 37 °C. In other embodiments, the compound degrades in a period of between about one hour and several weeks, depending on the desired application.

[0043] The terms "comprise," "comprising," "include," "including," "have," and "having" are used in the inclusive, open sense, meaning that additional elements may be included. The terms "such as", "e.g.", as used herein are non-limiting and are for illustrative purposes only. "Including" and "including but not limited to" are used interchangeably.

[0044] The term "drug delivery device" is an art-recognized term and refers to any medical device suitable for the application of a drug to a targeted organ or anatomic region. The term includes those devices that transport or accomplish the instillation of the compositions towards the targeted organ or anatomic area, even if the device itself is not formulated to include the composition. As an example, a needle or a catheter through which the composition is inserted into an anatomic area or into a blood vessel or other structure related to the anatomic area is understood to be a drug delivery device. As a further

example, a stent or a shunt or a catheter that has the composition included in its substance or coated on its surface is understood to be a drug delivery device.

[0045] When used with respect to a therapeutic agent or other material, the term "sustained release" is art-recognized. For example, a subject composition that releases a substance over time may exhibit sustained release characteristics, in contrast to a bolus type administration in which the entire amount of the substance is made biologically available at one time. For example, in particular embodiments, upon contact with body fluids including blood, tissue fluid, lymph or the like, the compound matrices (formulated as provided herein and otherwise as known to one of skill in the art) may undergo gradual degradation (e.g., through hydrolysis) with concomitant release of any material incorporated therein, for a sustained or extended period (as compared to the release from a bolus). This release may result in prolonged delivery of therapeutically effective amounts of any incorporated a therapeutic agent. Sustained release will vary in certain embodiments as described in greater detail below.

[0046] The term "delivery agent" is an art-recognized term, and includes molecules that facilitate the intracellular delivery of a therapeutic agent or other material. Examples of delivery agents include: sterols (e.g., cholesterol) and lipids (e.g., a cationic lipid, virosome or liposome).

[0047] The term "or" as used herein should be understood to mean "and/or", unless the context clearly indicates otherwise.

[0048] The phrases "parenteral administration" and "administered parenterally" are art-recognized terms, and include modes of administration other than enteral and topical administration, such as injections, and include, without limitation, intravenous, intramuscular, intrapleural, intravascular, intrapericardial, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intra-articular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

[0049] The term "treating" is art-recognized and includes inhibiting a disease, disorder or condition in a subject having been diagnosed with the disease, disorder, or condition, e.g., impeding its progress; and relieving the disease, disorder or condition, e.g., causing regression of the disease, disorder and/or condition. Treating the disease or condition includes ameliorating at least one symptom of the particular disease or condition, even if the underlying pathophysiology is not affected.

[0050] The term "preventing" is art-recognized and includes stopping a disease, disorder or condition from occurring in a subject which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it. Preventing a condition related to a disease includes stopping the condition from occurring after the disease has been diagnosed but before the condition has been diagnosed.

[0051] The term "fluid" is art-recognized to refer to a non-solid state of matter in which the atoms or molecules are free to move in relation to each other, as in a gas or liquid. If unconstrained upon application, a fluid material may flow to assume the shape of the space available to it, covering for example, the surfaces of an excisional site or the dead space left under a flap. A fluid material may be inserted or injected into a limited portion of a space and then may flow to enter a larger portion of the space or its entirety. Such a material may be termed "flowable." This term is art-recognized and includes, for example, liquid compositions that are capable of being sprayed into a site; injected with a manually operated syringe fitted with, for example, a 23-gauge needle; or delivered through a catheter. Also included in the term "flowable" are those highly viscous, "gel-like" materials at room temperature that may be delivered to the desired site by pouring, squeezing from a tube, or being injected with any one of the commercially available injection devices that provide injection pressures sufficient to propel highly viscous materials through a delivery system such as a needle or a catheter. When the compound used is itself flowable, a composition comprising it need not include a biocompatible solvent to allow its dispersion within a body cavity. Rather, the flowable compound may be delivered into the body cavity using a delivery system that relies upon the native flowability of the material for its application to the desired tissue surfaces. For example, if flowable, a composition comprising compounds can be injected to form, after injection, a temporary biomechanical barrier to coat or encapsulate internal organs or tissues, or it can be used to produce coatings for solid implantable devices. In certain instances, flowable subject compositions have the ability to assume, over time, the shape of the space containing it at body temperature.

[0052] Viscosity is understood herein as it is recognized in the art to be the internal friction of a fluid or the resistance to flow exhibited by a fluid material when subjected to deformation. The degree of viscosity of the compound may be adjusted by the molecular weight of the compound and other methods for altering the physical characteristics of a specific compound will be evident to practitioners of ordinary skill with no more than

routine experimentation. The molecular weight of the compound used may vary widely, depending on whether a rigid solid state (higher molecular weights) desirable, or whether a fluid state (lower molecular weights) is desired.

[0053] The phrase "pharmaceutically acceptable" is art-recognized. In certain embodiments, the term includes compositions, compounds and other materials and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0054] The phrase "pharmaceutically acceptable carrier" is art-recognized, and includes, for example, pharmaceutically acceptable materials, compositions or vehicles, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any subject composition from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of a subject composition and not injurious to the patient. In certain embodiments, a pharmaceutically acceptable carrier is non-pyrogenic. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

[0055] The term "pharmaceutically acceptable salts" is art-recognized, and includes relatively non-toxic, inorganic and organic acid addition salts of compositions, including without limitation, therapeutic agents, excipients, other materials and the like. Examples of pharmaceutically acceptable salts include those derived from mineral acids, such as hydrochloric acid and sulfuric acid, and those derived from organic acids, such as

ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, and the like. Examples of suitable inorganic bases for the formation of salts include the hydroxides, carbonates, and bicarbonates of ammonia, sodium, lithium, potassium, calcium, magnesium, aluminum, zinc and the like. Salts may also be formed with suitable organic bases, including those that are non-toxic and strong enough to form such salts. For purposes of illustration, the class of such organic bases may include mono-, di-, and trialkylamines, such as methylamine, dimethylamine, and triethylamine; mono-, di- or trihydroxyalkylamines such as mono-, di-, and triethanolamine; amino acids, such as arginine and lysine; guanidine; N-methylglucosamine; N-methylglucamine; L-glutamine; N-methylpiperazine; morpholine; ethylenediamine; N-benzylphenethylamine; (trihydroxymethyl)aminoethane; and the like. See, for example, J. Pharm. Sci., 66:1-1 9 (1977).

[0056] A "patient," "subject," or "host" to be treated by the subject method may mean either a human or non-human animal, such as primates, mammals, and vertebrates.

[0057] The term "prophylactic or therapeutic" treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, i.e., it protects the host against developing the unwanted condition, whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

[0058] The terms "therapeutic agent", "drug", "medicament" and "bioactive substance" are art-recognized and include molecules and other agents that are biologically, physiologically, or pharmacologically active substances that act locally or systemically in a patient or subject to treat a disease or condition, such as macular degeneration. The terms include without limitation pharmaceutically acceptable salts thereof and pro-drugs. Such agents may be acidic, basic, or salts; they may be neutral molecules, polar molecules, or molecular complexes capable of hydrogen bonding; they may be prodrugs in the form of ethers, esters, amides and the like that are biologically activated when administered into a patient or subject.

[0059] The phrase "therapeutically effective amount" is an art-recognized term. In certain embodiments, the term refers to an amount of a therapeutic agent that, when incorporated into a compound, produces some desired effect at a reasonable benefit/risk ratio applicable to any medical treatment. In certain embodiments, the term refers to that amount necessary

or sufficient to eliminate, reduce or maintain (e.g., prevent the spread of) a tumor or other target of a particular therapeutic regimen. The effective amount may vary depending on such factors as the disease or condition being treated, the particular targeted constructs being administered, the size of the subject or the severity of the disease or condition. One of ordinary skill in the art may empirically determine the effective amount of a particular compound without necessitating undue experimentation. In certain embodiments, a therapeutically effective amount of a therapeutic agent for *in vivo* use will likely depend on a number of factors, including: the rate of release of an agent from a compound matrix, which will depend in part on the chemical and physical characteristics of the compound; the identity of the agent; the mode and method of administration; and any other materials incorporated in the compound matrix in addition to the agent.

[0060] "Radiosensitizer" is defined as a therapeutic agent that, upon administration in a therapeutically effective amount, promotes the treatment of one or more diseases or conditions that are treatable with electromagnetic radiation. In general, radiosensitizers are intended to be used in conjunction with electromagnetic radiation as part of a prophylactic or therapeutic treatment. Appropriate radiosensitizers to use in conjunction with treatment with the subject compositions will be known to those of skill in the art.

[0061] "Electromagnetic radiation" as used in this specification includes, but is not limited to, radiation having the wavelength of 10^{-20} to 10 meters. Particular embodiments of electromagnetic radiation employ the electromagnetic radiation of: gamma-radiation (10^{-20} to 10^{-13} m), x-ray radiation (10^{-11} to 10^{-9} m), ultraviolet light (10 nm to 400 nm), visible light (400 nm to 700 nm), infrared radiation (700 nm to 1.0 mm), and microwave radiation (1 mm to 30 cm).

[0062] The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" are art-recognized, and include the administration of a subject composition or other material at a site remote from the site affected by the disease being treated. Administration of an agent directly into, onto or in the vicinity of a lesion of the disease being treated, even if the agent is subsequently distributed systemically, may be termed "local" or "topical" or "regional" administration.

[0063] The term "ED_{50}" is art-recognized. In certain embodiments, ED_{50} means the dose of a drug which produces 50% of its maximum response or effect, or alternatively, the dose which produces a pre-determined response in 50% of test subjects or preparations. The term "LD_{50}" is art-recognized. In certain embodiments, LD_{50} means the dose of a drug which is

lethal in 50% of test subjects. The term "therapeutic index" is an art-recognized term which refers to the therapeutic index of a drug, defined as LD_{50}/ED_{50}.

[0064] The terms "incorporated" and "encapsulated" are art-recognized when used in reference to a therapeutic agent and a compound, such as a composition disclosed herein. In certain embodiments, these terms include incorporating, formulating or otherwise including such agent into a composition which allows for sustained release of such agent in the desired application. The terms may contemplate any manner by which a therapeutic agent or other material is incorporated into a compound matrix, including for example: the compound is a polymer, and the agent is attached to a monomer of such polymer (by covalent or other binding interaction) and having such monomer be part of the polymerization to give a polymeric formulation, distributed throughout the polymeric matrix, appended to the surface of the polymeric matrix (by covalent or other binding interactions), encapsulated inside the polymeric matrix, etc. The term "co-incorporation" or "co-encapsulation" refers to the incorporation of a therapeutic agent or other material and at least one other a therapeutic agent or other material in a subject composition.

[0065] More specifically, the physical form in which a therapeutic agent or other material is encapsulated in compounds may vary with the particular embodiment. For example, a therapeutic agent or other material may be first encapsulated in a microsphere and then combined with the compound in such a way that at least a portion of the microsphere structure is maintained. Alternatively, a therapeutic agent or other material may be sufficiently immiscible in a controlled-release compound that it is dispersed as small droplets, rather than being dissolved, in the compound. Any form of encapsulation or incorporation is contemplated by the present disclosure, in so much as the sustained release of any encapsulated therapeutic agent or other material determines whether the form of encapsulation is sufficiently acceptable for any particular use.

[0066] The term "biocompatible plasticizer" is art-recognized, and includes materials which are soluble or dispersible in the controlled-release compositions described herein, which increase the flexibility of the compound matrix, and which, in the amounts employed, are biocompatible. Suitable plasticizers are well known in the art and include those disclosed in U.S. Patent Nos. 2,784,127 and 4,444,933. Specific plasticizers include, by way of example, acetyl tri-n-butyl citrate (about 20 weight percent or less), acetyl trihexyl citrate (about 20 weight percent or less), butyl benzyl phthalate, dibutyl phthalate,

dioctylphthalate, n-butyryl tri-n-hexyl citrate, diethylene glycol dibenzoate (c. 20 weight percent or less) and the like.

[0067] "Small molecule" is an art-recognized term. In certain embodiments, this term refers to a molecule which has a molecular weight of less than about 2000 amu, or less than about 1000 amu, and even less than about 500 amu.

[0068] The term "alkyl" is art-recognized, and includes saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In certain embodiments, a straight chain or branched chain alkyl has about 30 or fewer carbon atoms in its backbone (e.g., C1-C30 for straight chain, C3-C30 for branched chain), and alternatively, about 20 or fewer. Likewise, cycloalkyls have from about 3 to about 10 carbon atoms in their ring structure, and alternatively about 5, 6 or 7 carbons in the ring structure.

[0069] Unless the number of carbons is otherwise specified, "lower alkyl" refers to an alkyl group, as defined above, but having from one to about ten carbons, alternatively from one to about six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths.

[0070] The term "aralkyl" is art-recognized and refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

[0071] The terms "alkenyl" and "alkynyl" are art-recognized and refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

[0072] The term "aryl" is art-recognized and refers to 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, naphthalene, anthracene, pyrene, pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring may be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, -CF_3, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or

more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings may be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

[0073] The terms ortho, meta and para are art-recognized and refer to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and ortho-dimethylbenzene are synonymous.

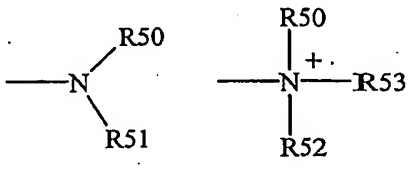
[0074] The terms "heterocyclyl", "heteroaryl", or "heterocyclic group" are art-recognized and refer to 3- to about 10-membered ring structures, alternatively 3- to about 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles may also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxanthene, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring may be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

[0075] The terms "polycyclyl" or "polycyclic group" are art-recognized and refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle may be substituted with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

[0076] The term "carbocycle" is art-recognized and refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

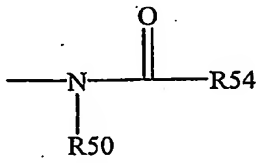
[0077] The term "nitro" is art-recognized and refers to $-NO_2$; the term "halogen" is art-recognized and refers to -F, -Cl, -Br or -I; the term "sulfhydryl" is art-recognized and refers to SH; the term "hydroxyl" means -OH; and the term "sulfonyl" is art-recognized and refers to SO_2^-. "Halide" designates the corresponding anion of the halogens, and "pseudohalide" has the definition set forth on page 560 of "Advanced Inorganic Chemistry" by Cotton and Wilkinson.

[0078] The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that may be represented by the general formulas:



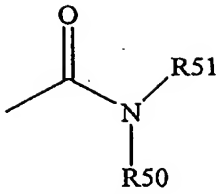
[0079] wherein R50, R51 and R52 each independently represent a hydrogen, an alkyl, an alkenyl, $(CH_2)_m$-R61, or R50 and R51, taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R61 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In other embodiments, R50 and R51 (and optionally R52) each independently represent a hydrogen, an alkyl, an alkenyl, or $-(CH_2)_m$-R61. Thus, the term "alkylamine" includes an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R50 and R51 is an alkyl group.

[0080] The term "acylamino" is art-recognized and refers to a moiety that may be represented by the general formula:



[0081] wherein R50 is as defined above, and R54 represents a hydrogen, an alkyl, an alkenyl or $-(CH_2)_m$-R61, where m and R61 are as defined above.

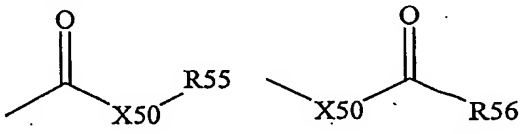
[0082] The term "amido" is art recognized as an amino-substituted carbonyl and includes a moiety that may be represented by the general formula:



[0083] wherein R50 and R51 are as defined above. Certain embodiments of the amide in the present invention will not include imides which may be unstable.

[0084] The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In certain embodiments, the "alkylthio" moiety is represented by one of S alkyl, -S-alkenyl, -S-alkynyl, and -S-$(CH_2)_m$-R61, wherein m and R61 are defined above. Representative alkylthio groups include methylthio, ethyl thio, and the like.

[0085] The term "carboxyl" is art recognized and includes such moieties as may be represented by the general formulas:

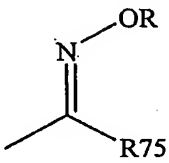


[0086] wherein X50 is a bond or represents an oxygen or a sulfur, and R55 and R56 represents a hydrogen, an alkyl, an alkenyl, -$(CH_2)_m$-R61or a pharmaceutically acceptable salt, R56 represents a hydrogen, an alkyl, an alkenyl or -$(CH_2)_m$-R61, where m and R61 are defined above. Where X50 is an oxygen and R55 or R56 is not hydrogen, the formula represents an "ester". Where X50 is an oxygen, and R55 is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R55 is a hydrogen, the formula represents a "carboxylic acid". Where X50 is an oxygen, and R56 is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiolcarbonyl" group. Where X50 is a sulfur and R55 or R56 is not hydrogen, the formula represents a "thiolester." Where X50 is a sulfur and R55 is hydrogen, the formula represents a "thiolcarboxylic acid." Where X50 is a sulfur and R56 is hydrogen, the formula represents a "thiolformate." On the other hand, where X50 is a bond, and R55 is not hydrogen, the above formula represents a "ketone" group. Where X50 is a bond, and R55 is hydrogen, the above formula represents an "aldehyde" group.

[0087] The term "carbamoyl" refers to -O(C=O)NRR', where R and R' are independently H, aliphatic groups, aryl groups or heteroaryl groups.

[0088] The term "oxo" refers to a carbonyl oxygen (=O).

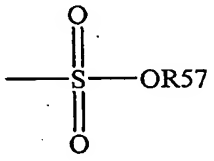
[0089] The terms "oxime" and "oxime ether" are art-recognized and refer to moieties that may be represented by the general formula:



[0090] wherein R75 is hydrogen, alkyl, cycloalkyl, alkenyl, alkynyl, aryl, aralkyl, or $-(CH_2)_m$-R61. The moiety is an "oxime" when R is H; and it is an "oxime ether" when R is alkyl, cycloalkyl, alkenyl, alkynyl, aryl, aralkyl, or $-(CH_2)_m$-R61.

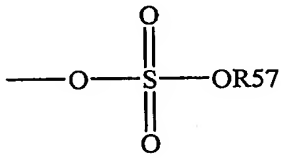
[0091] The terms "alkoxyl" or "alkoxy" are art-recognized and refer to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as may be represented by one of -O-alkyl, -O-alkenyl, O-alkynyl, $-O-(CH_2)_m$-R61, where m and R61 are described above.

[0092] The term "sulfonate" is art recognized and refers to a moiety that may be represented by the general formula:



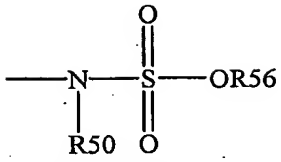
[0093] in which R57 is an electron pair, hydrogen, alkyl, cycloalkyl, or aryl.

[0094] The term "sulfate" is art recognized and includes a moiety that may be represented by the general formula:



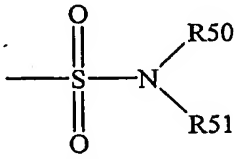
[0095] in which R57 is as defined above.

[0096] The term "sulfonamido" is art recognized and includes a moiety that may be represented by the general formula:



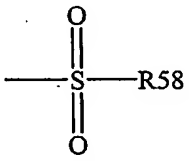
[0097] in which R50 and R56 are as defined above.

[0098] The term "sulfamoyl" is art-recognized and refers to a moiety that may be represented by the general formula:



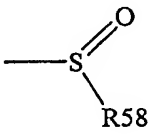
[0099] in which R50 and R51 are as defined above.

[0100] The term "sulfonyl" is art-recognized and refers to a moiety that may be represented by the general formula:



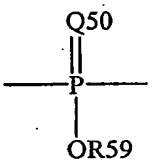
[0101] in which R58 is one of the following: hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl or heteroaryl.

[0102] The term "sulfoxido" is art-recognized and refers to a moiety that may be represented by the general formula:

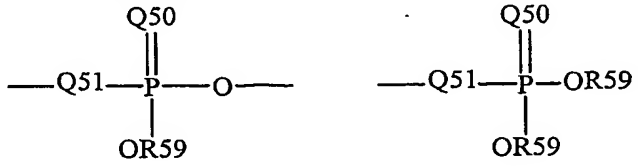


[0103] in which R58 is defined above.

[0104] The term "phosphoryl" is art-recognized and may in general be represented by the formula:

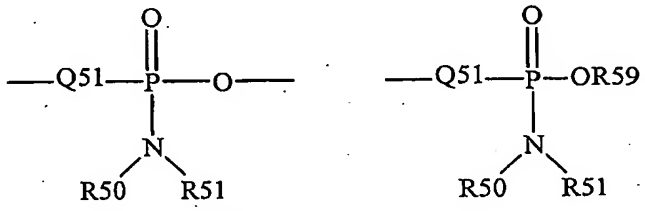


[0105] wherein Q50 represents S or O, and R59 represents hydrogen, a lower alkyl or an aryl. When used to substitute, e.g., an alkyl, the phosphoryl group of the phosphorylalkyl may be represented by the general formulas:



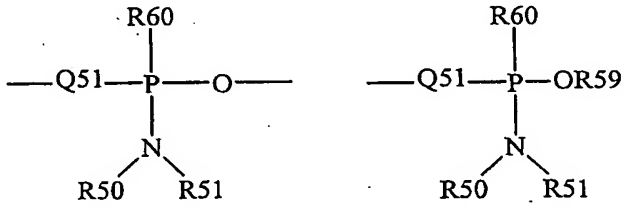
[0106] wherein Q50 and R59, each independently, are defined above, and Q51 represents O, S or N. When Q50 is S, the phosphoryl moiety is a "phosphorothioate".

[0107] The term "phosphoramidite" is art-recognized and may be represented in the general formulas:



[0108] wherein Q51, R50, R51 and R59 are as defined above.

[0109] The term "phosphonamidite" is art-recognized and may be represented in the general formulas:



[0110] wherein Q51, R50, R51 and R59 are as defined above, and R60 represents a lower alkyl or an aryl.

[0111] Analogous substitutions may be made to alkenyl and alkynyl groups to produce, for example, aminoalkenyls, aminoalkynyls, amidoalkenyls, amidoalkynyls, iminoalkenyls, iminoalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

[0112] The definition of each expression, e.g. alkyl, m, n, and the like, when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

[0113] The term "selenoalkyl" is art-recognized and refers to an alkyl group having a substituted seleno group attached thereto. Exemplary "selenoethers" which may be

substituted on the alkyl are selected from one of -Se-alkyl, -Se-alkenyl, -Se-alkynyl, and -Se-$(CH_2)_m$-R61, m and R61 being defined above.

[0114] The terms triflyl, tosyl, mesyl, and nonaflyl are art-recognized and refer to trifluoromethanesulfonyl, p-toluenesulfonyl, methanesulfonyl, and nonafluorobutanesulfonyl groups, respectively. The terms triflate, tosylate, mesylate, and nonaflate are art-recognized and refer to trifluoromethanesulfonate ester, p-toluenesulfonate ester, methanesulfonate ester, and nonafluorobutanesulfonate ester functional groups and molecules that contain said groups, respectively.

[0115] The abbreviations Me, Et, Ph, Tf, Nf, Ts, and Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, p-toluenesulfonyl and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the Journal of Organic Chemistry; this list is typically presented in a table entitled Standard List of Abbreviations.

[0116] Certain compounds contained in compositions of the present invention may exist in particular geometric or stereoisomeric forms. In addition, polymers of the present invention may also be optically active. The present invention contemplates all such compounds, including cis- and trans-isomers, R- and S-enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

[0117] If, for instance, a particular enantiomer of compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

[0118] It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted

atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction.

[0119] The term "substituted" is also contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents may be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

[0120] 3. Compositions

[0121] As described above, macular degeneration may be treated or prevented by interfering with the visual cycle in such a way that diminishes the amount of all-*trans*-retinal present in the discs of the rod photoreceptor outer segments. Production of retinotoxic compounds by cone cells is negligible and may be ignored, because rods represent 95% of all photoreceptors.

[0122] Figure 1 depicts the mammalian visual cycle. In the course of the visual cycle, a complex of 11-*cis*-retinal and opsin, known as rhodopsin, passes through a series of biochemical steps initiated by the absorption of light. Various steps of this cycle in distinct places. As Figure 1 illustrates, the initial steps of light absorption to the dissociation of opsin and the formation of all-*trans*-retinal occur in the discs of the rod photoreceptor cell outer segment. The reduction of all-*trans*-retinal to all-*trans*-retinol takes place in the cytoplasm of the rod cell, and the remaining steps to regenerate 11-*cis*-retinal occur in the retinal pigment epithelium (RPE).

[0123] At least two broad approaches are contemplated for preventing the accumulation of all-*trans*-retinal in the disc. In one approach, one or more enzymatic steps or chaperone binding steps in the visual cycle may be inhibited so that the synthetic pathway to all-*trans*-retinal is blocked. In another approach, a portion of the visual cycle is "short-circuited," i.e., an early intermediate in the cycle is shunted to an intermediate that is two or more steps

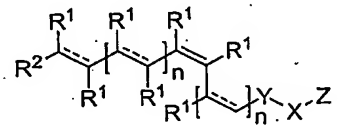
later in the visual cycle, so that these steps of the cycle are bypassed while the all-*trans*-retinal precursors are not in the disc.

[0124] A. Enzyme inhibitors

[0125] Limiting the flux of retinoids through the visual cycle can be achieved by inhibiting any of the key biochemical reactions of the visual cycle. Each step of the cycle is potentially addressable in this fashion. Inhibiting an enzymatic step could thus be used to "stall" the visual cycle in the RPE, thereby keeping all-*trans*-retinal out of the discs.

[0126] Other steps in the visual cycle are also prone to inhibition. For example, as shown in Figure 1, several enzymes act upon all-*trans*-retinol and its derivatives upon its return to the RPE, including LRAT (lecithin retinol acyl transferase), 11-*cis*-retinol dehydrogenase and IMH (isomerohydrolase). In addition, the chaperone RPE65 binds retinyl esters to make those typically hydrophobic compounds available to IMH for processing to 11-*cis*-retinol. These enzymes and chaperone may be targeted for inhibition and/or interference.

[0127] In certain embodiments, an inhibitor of isomerohydrolase (IMH), an inhibitor 11-*cis*-retinol dehydrogenase, an inhibitor of lecithin retinol acyl transferase (LRAT), or an antagonist of chaperone retinal pigment epithelium (RPE65) has a structure represented by formula I:



I

wherein, independently for each occurrence,

n is 0 to 10 inclusive;

R^1 is hydrogen or alkyl;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

Y is $-C(R_b)_p-$, $-C(=O)-$ or $-C(R_b)_pC(=O)-$;

X is $-O-$, $-N(R_a)-$, $-C(R_b)_p-$ or $-S-$;

Z is alkyl, haloalkyl, $-(CH_2CH_2O)_pR_b$ or $-C(=O)R_b$;

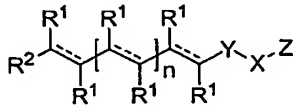
p is 0 to 20 inclusive;

R_a is hydrogen, alkyl, aryl or aralkyl;

R_b is hydrogen, alkyl or haloalkyl; and

=== denotes a single bond, a *cis* double bond, or a *trans* double bond.

[0128] In certain embodiments, an inhibitor of isomerohydrolase (IMH), an inhibitor 11-*cis*-retinol dehydrogenase, an inhibitor of lecithin retinol acyl transferase (LRAT), or an antagonist of chaperone retinal pigment epithelium (RPE65) has a structure represented by formula **II**:



II

wherein

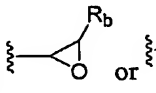
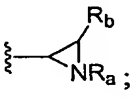
n is 0 to 10 inclusive;

R^1 is hydrogen or alkyl;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

Y is $-C(R_b)_p-$, $-C(=O)-$ or $-C(R_b)_pC(=O)-$;

X is hydrogen, -O-, -S-, $-N(R_a)-$, $-N(R_a)-N(R_a)-$, $-C(=O)-$, $-C(=NR_a)-$, -C(=NOH)-, -C(=S)- or $-C(R_b)_p-$;

Z is absent, hydrogen, alkyl, haloalkyl, aryl, aralkyl, -CN, $-OR_b$, $-(CH_2CH_2O)_pR_b$, $-C(=O)R_b$, $-C(=O)CH_2F$, $-C(=O)CHF_2$, $-C(=O)CF_3$, $-C(=O)CHN_2$, $-C(=O)OR_b$, $-C(=O)CH_2OC(=O)R_b$, $-C(=O)C(=C(R_b)_2)R_b$,  or  ;

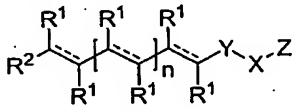
p is 0 to 20 inclusive;

R_a is hydrogen, alkyl, aryl or aralkyl;

R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl; and

⎓ denotes a single bond, a *cis* double bond or a *trans* double bond.

[0129] In certain embodiments, an inhibitor of isomerohydrolase (IMH), an inhibitor 11-*cis*-retinol dehydrogenase, an inhibitor of lecithin retinol acyl transferase (LRAT), or an antagonist of chaperone retinal pigment epithelium (RPE65) has a structure represented by formula **III**:



III

wherein

n is 0 to 10 inclusive;

R^1 is hydrogen or alkyl;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

Y is $-CR_b(OR_b)-$, $-CR_b(N(R_a)_2)-$, $-C(R_b)_p-$, $-C(=O)-$ or $-C(R_b)_pC(=O)-$;

X is $-O-$, $-S-$, $-N(R_a)-$, $-C(=O)-$, or $-C(R_b)_p-$;

Z is hydrogen, alkyl, haloalkyl, aryl, aralkyl, $-OR_b$, $-N(R_b)_2$, $-(CH_2CH_2O)_pR_b$, $-C(=O)R_b$, $-C(=NR_a)R_b$, $-C(=NOR_b)R_b$, $-C(OR_b)(R_b)_2$, $-C(N(R_a)_2)(R_b)_2$ or $-(CH_2CH_2O)_pR_b$;

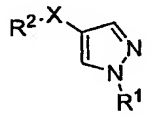
p is 0 to 20 inclusive;

R_a is hydrogen, alkyl, aryl or aralkyl;

R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl; and

≡ denotes a single bond or a *trans* double bond.

[0130] In certain embodiments, an inhibitor of isomerohydrolase (IMH), an inhibitor 11-*cis*-retinol dehydrogenase, an inhibitor of lecithin retinol acyl transferase (LRAT), or an antagonist of chaperone retinal pigment epithelium (RPE65) has a structure represented by formula **VI**:



VI

wherein, independently for each occurrence,

R^1 is hydrogen, alkyl, aryl or aralkyl;

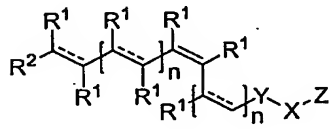
X is alkyl, alkenyl, $-C(R_b)_2-$, $-C(=O)-$, $-C(=NR_a)-$, $-C(OH)R_b$ or $-C(N(R_a)_2)R_b-$;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

R_a is hydrogen, alkyl, aryl or aralkyl; and

R_b is hydrogen or alkyl.

[0131] In certain embodiments, an inhibitor of isomerohydrolase (IMH), an inhibitor 11-*cis*-retinol dehydrogenase, an inhibitor of lecithin retinol acyl transferase (LRAT), or an antagonist of chaperone retinal pigment epithelium (RPE65) has a structure represented by formula **I**:



I

wherein, independently for each occurrence,

n is 0 to 10 inclusive;

R^1 is hydrogen or alkyl;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

Y is $-C(R_b)_p-$, $-C(=O)-$ or $-C(R_b)_pC(=O)-$;

X is $-O-$, $-N(R_a)-$, $-C(R_b)_p-$ or $-S-$;

Z is alkyl, haloalkyl, $-(CH_2CH_2O)_pR_b$ or $-C(=O)R_b$;

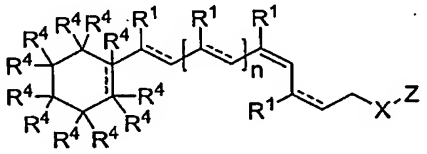
p is 0 to 20 inclusive;

R_a is hydrogen, alkyl, aryl or aralkyl;

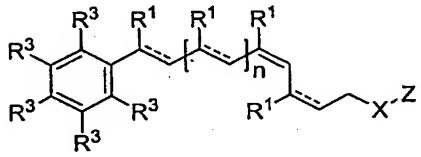
R_b is hydrogen, alkyl or haloalkyl; and

=== denotes a single bond, a *cis* double bond, or a *trans* double bond.

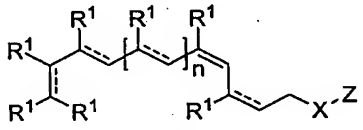
[0132] In certain embodiments, an inhibitor of isomerohydrolase (IMH) has a structure represented by formula **Ia**, **Ib**, **Ic**, or **Id**:



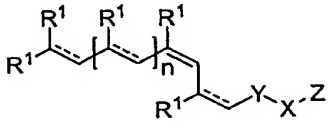
Ia



Ib



Ic



Id

wherein, independently for each occurrence,

n is 0 to 4 inclusive;

R^1 is hydrogen or alkyl;

R^3 is hydrogen, halogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, aralkyenyl, aralkynyl, heteroaralkyl, heteroaralkyenyl, heteroaralkynyl, cyano, nitro, sulfhydryl, hydroxyl, sulfonyl, amino, acylamino, amido, alkylthio, carboxyl, carbamoyl, alkoxyl, sulfonate, sulfate, sulfonamido, sulfamoyl, sulfonyl, and sulfoxido;

R^4 is absent, hydrogen, halogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, aralkyenyl, aralkynyl, heteroaralkyl, heteroaralkyenyl, heteroaralkynyl, cyano, nitro, sulfhydryl, hydroxyl, sulfonyl, amino, acylamino, amido, alkylthio, carboxyl, carbamoyl, alkoxyl, sulfonate, sulfate, sulfonamido, sulfamoyl, sulfonyl, and sulfoxido;

Y is $-C(R_b)_2-$ or $-C(=O)-$;

X is $-O-$, $-N(R_a)-$, $-C(R_b)_2-$ or $-S-$;

Z is alkyl, haloalkyl or $-C(=O)R_b$;

R_a is hydrogen, alkyl, aryl or aralkyl;

R_b is hydrogen, alkyl or haloalkyl; and

≡≡≡ denotes a single bond, a *cis* double bond, or a *trans* double bond.

[0133] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein R^1 is methyl.

[0134] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein n is 0.

[0135] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein n is 1.

[0136] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein Y is $-CH_2-$.

[0137] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein X is -O-.

[0138] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein X is -N(H)-.

[0139] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein Z is $-C(=O)R_b$.

[0140] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein Z is $-C(=O)R_b$; and R_b is haloalkyl.

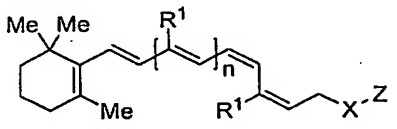
[0141] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein Z is alkyl.

[0142] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein Z is haloalkyl.

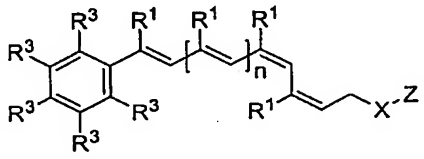
[0143] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein R^3 is hydrogen.

[0144] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ia**, **Ib**, **Ic**, or **Id**, wherein R^4 is hydrogen, methyl or absent.

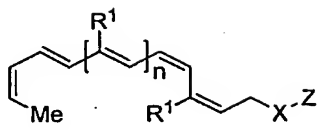
[0145] In certain embodiments, an inhibitor of isomerohydrolase (IMH) has a structure represented by formula **Ie**, **If**, **Ig**, or **Ih**:



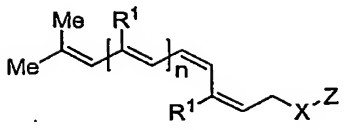
Ie



If



Ig



Ih

wherein, independently for each occurrence,

n is 0 to 4 inclusive;

R^1 is hydrogen or alkyl;

R^3 is hydrogen, halogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, aralkyenyl, aralkynyl, heteroaralkyl, heteroaralkyenyl, heteroaralkynyl, cyano, nitro, sulfhydryl, hydroxyl, sulfonyl, amino, acylamino, amido, alkylthio, carboxyl, carbamoyl, alkoxyl, sulfonate, sulfate, sulfonamido, sulfamoyl, sulfonyl, and sulfoxido;

X is -O-, -N(R_a)-, -C(R_b)$_2$- or -S-;

Z is alkyl, haloalkyl or -C(=O)R_b;

R_a is hydrogen, alkyl, aryl or aralkyl; and

R_b is hydrogen, alkyl or haloalkyl.

[0146] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein n is 0.

[0147] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein n is 1.

[0148] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein X is -O-.

[0149] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein X is -N(H)-.

[0150] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein Z is -C(=O)R_b.

[0151] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein Z is -C(=O)R_b; and R_b is haloalkyl.

[0152] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein Z is alkyl.

[0153] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein Z is haloalkyl.

[0154] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein R^3 is hydrogen.

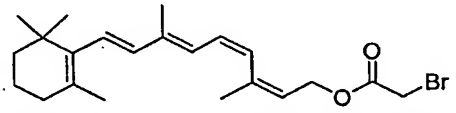
[0155] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein X is -O-; and Z is alkyl.

[0156] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein X is -O-; and Z is haloalkyl.

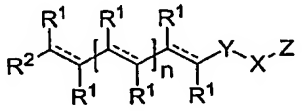
[0157] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein X is -N(H)-; and Z is alkyl.

[0158] In further embodiments, an inhibitor of isomerohydrolase (IMH) has the structure of formula **Ie**, **If**, **Ig**, or **Ih**, wherein X is -N(H)-; and Z is haloalkyl.

[0159] In one embodiment, an inhibitor of isomerohydrolase (IMH) is 11-*cis*-retinyl bromoacetate (cBRA):



[0160] In certain embodiments, an inhibitor of isomerohydrolase (IMH), an inhibitor 11-*cis*-retinol dehydrogenase, an inhibitor of lecithin retinol acyl transferase (LRAT), or an antagonist of chaperone retinal pigment epithelium (RPE65) has a structure represented by formula **II**:



II

wherein

n is 0 to 10 inclusive;

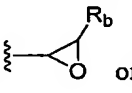
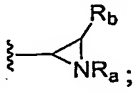
R^1 is hydrogen or alkyl;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

Y is $-C(R_b)_p-$, -C(=O)- or $-C(R_b)_pC(=O)-$;

X is hydrogen, -O-, -S-, $-N(R_a)-$, $-N(R_a)-N(R_a)-$, -C(=O)-, $-C(=NR_a)-$, -C(=NOH)-, -C(=S)- or $-C(R_b)_p-$;

Z is absent, hydrogen, alkyl, haloalkyl, aryl, aralkyl, -CN, $-OR_b$, $-(CH_2CH_2O)_pR_b$, $-C(=O)R_b$, $-C(=O)CH_2F$, $-C(=O)CHF_2$, $-C(=O)CF_3$, $-C(=O)CHN_2$, $-C(=O)OR_b$,

$-C(=O)CH_2OC(=O)R_b$, $-C(=O)C(=C(R_b)_2)R_b$,  or  ;

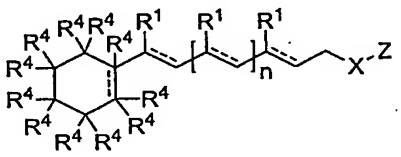
p is 0 to 20 inclusive;

R_a is hydrogen, alkyl, aryl or aralkyl;

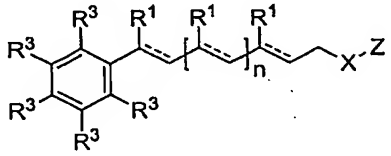
R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl; and

≡ denotes a single bond, a *cis* double bond or a *trans* double bond.

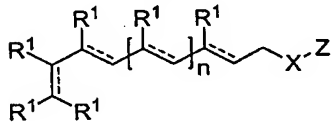
[0161] In certain embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa**, **IIb**, **IIc**, or **IId**:



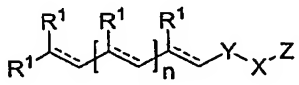
IIa



IIb



IIc



IId

wherein, independently for each occurrence,

n is 0 to 4 inclusive;

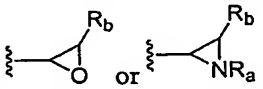
R^1 is hydrogen or alkyl;

R^3 is hydrogen, halogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, aralkyenyl, aralkynyl, heteroaralkyl, heteroaralkyenyl, heteroaralkynyl, cyano, nitro, sulfhydryl, hydroxyl, sulfonyl, amino, acylamino, amido, alkylthio, carboxyl, carbamoyl, alkoxyl, sulfonate, sulfate, sulfonamido, sulfamoyl, sulfonyl, and sulfoxido;

R^4 is absent, hydrogen, halogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, aralkyenyl, aralkynyl, heteroaralkyl, heteroaralkyenyl, heteroaralkynyl, cyano, nitro, sulfhydryl, hydroxyl, sulfonyl, amino, acylamino, amido, alkylthio, carboxyl, carbamoyl, alkoxyl, sulfonate, sulfate, sulfonamido, sulfamoyl, sulfonyl, and sulfoxido;

Y is $-C(=O)-$ or $-C(R_b)_2-$;

X is hydrogen, -O-, -S-, $-N(R_a)-$, $-N(R_a)-N(R_a)-$, $-C(=O)-$, $-C(=NR_a)-$, -C(=NOH)-, -C(=S)- or $-C(R_b)_2-$;

Z is absent, hydrogen, alkyl, haloalkyl, aryl, aralkyl, -CN, $-OR_b$, $-C(=O)R_b$, $-C(=O)CH_2F$, $-C(=O)CHF_2$, $-C(=O)CF_3$, $-C(=O)CHN_2$, $-C(=O)CH_2OC(=O)R_b$, $-C(=O)OR_b$, $-C(=O)C(=C(R_b)_2)R_b$,  ;

R_a is hydrogen, alkyl, aryl or aralkyl;

R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl; and

≡ denotes a single bond, a *cis* double bond or a *trans* double bond.

[0162] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein n is 0.

[0163] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein n is 1.

[0164] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein R^1 is hydrogen or methyl.

[0165] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein R^3 is hydrogen.

[0166] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein R^4 is hydrogen or methyl.

[0167] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein Y is $-CH_2-$

[0168] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein X is -O-.

[0169] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein X is -NH-.

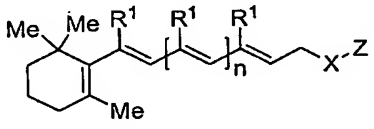
[0170] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein X is $-C(R_b)_2-$.

[0171] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein X is -C(=O)-.

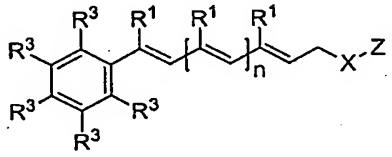
[0172] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein Z is alkyl.

[0173] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIa, IIb, IIc,** or **IId,** wherein Z is haloalkyl.

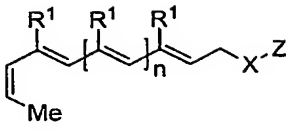
[0174] In certain embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh:**



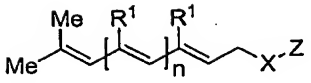
IIe



IIf



IIg



IIh

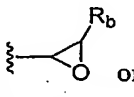
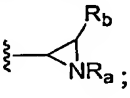
wherein, independently for each occurrence,

n is 0 to 4 inclusive;

R^1 is hydrogen or alkyl;

R^3 is hydrogen, halogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, aralkyenyl, aralkynyl, heteroaralkyl, heteroaralkyenyl, heteroaralkynyl, cyano, nitro, sulfhydryl, hydroxyl, sulfonyl, amino, acylamino, amido, alkylthio, carboxyl, carbamoyl, alkoxyl, sulfonate, sulfate, sulfonamido, sulfamoyl, sulfonyl, and sulfoxido;

X is hydrogen, -O-, -S-, -N(R_a)-, -N(R_a)-N(R_a)-, -C(=O)-, -C(=NR_a)-, -C(=NOH)-, -C(=S)- or -C(R_b)$_2$-;

Z is absent, hydrogen, alkyl, haloalkyl, aryl, aralkyl, -CN, -OR_b, -C(=O)R_b, -C(=O)CH_2F, -C(=O)CHF_2, -C(=O)CF_3, -C(=O)CHN_2, -C(=O)CH_2OC(=O)R_b, -C(=O)OR_b, -C(=O)C(=C(R_b)$_2$)R_b,  or  ;

R_a is hydrogen, alkyl, aryl or aralkyl; and

R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl.

[0175] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein n is 0.

[0176] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein n is 1.

[0177] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein R^1 is hydrogen or methyl.

[0178] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein R^3 is hydrogen.

[0179] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein R^4 is hydrogen or methyl.

[0180] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein X is -O-.

[0181] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein X is -NH-.

[0182] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein X is $-CH_2-$.

[0183] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein X is -C(=O)-.

[0184] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein Z is alkyl.

[0185] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein Z is haloalkyl.

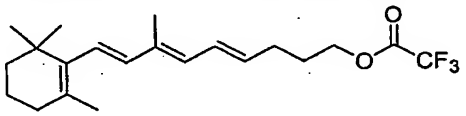
[0186] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein Z is $-C(=O)R_b$.

[0187] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein X is -O-; and Z is $-C(=O)R_b$.

[0188] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein X is $-CH_2-$; and Z is $-C(=O)R_b$.

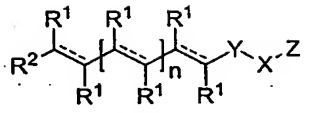
[0189] In further embodiments, an inhibitor of lecithin retinol acyl transferase (LRAT) has a structure represented by formula **IIe, IIf, IIg,** or **IIh,** wherein X is -NH-; and Z is $-C(=O)R_b$.

[0190] In one embodiment, an inhibitor of lecithin retinol acyl transferase (LRAT) is 13-desmethyl-13,14-dihydro-all-*trans*-retunyl trifluoroacetate (RFA):



[0191] In one embodiment, an inhibitor of lecithin retinol acyl transferase (LRAT) is all-*trans*-retinyl α-bromoacetate.

[0192] In certain embodiments, an inhibitor of isomerohydrolase (IMH), an inhibitor 11-*cis*-retinol dehydrogenase, an inhibitor of lecithin retinol acyl transferase (LRAT), or an antagonist of chaperone retinal pigment epithelium (RPE65) has a structure represented by formula **III:**



III

wherein

n is 0 to 10 inclusive;

R^1 is hydrogen or alkyl;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

Y is $-CR_b(OR_b)-$, $-CR_b(N(R_a)_2)-$, $-C(R_b)_p-$, $-C(=O)-$ or $-C(R_b)_pC(=O)-$;

X is $-O-$, $-S-$, $-N(R_a)-$, $-C(=O)-$, or $-C(R_b)_p-$;

Z is hydrogen, alkyl, haloalkyl, aryl, aralkyl, $-OR_b$, $-N(R_b)_2$, $-(CH_2CH_2O)_pR_b$, $-C(=O)R_b$, $-C(=NR_a)R_b$, $-C(=NOR_b)R_b$, $-C(OR_b)(R_b)_2$, $-C(N(R_a)_2)(R_b)_2$ or $-(CH_2CH_2O)_pR_b$;

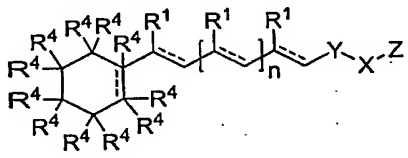
p is 0 to 20 inclusive;

R_a is hydrogen, alkyl, aryl or aralkyl;

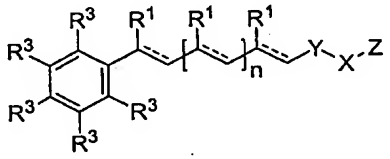
R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl; and

=== denotes a single bond or a *trans* double bond.

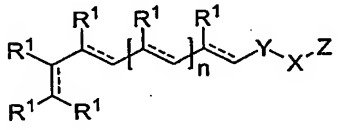
[0193] In certain embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc** or **IIId**:



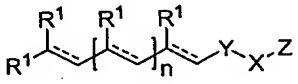
IIIa



IIIb



IIIc



IIId

wherein, independently for each occurrence,

n is 0 to 4 inclusive;

R^1 is hydrogen or alkyl;

Y is $-C(=O)-$, $-CR_b(OR_b)-$, $-CR_b(N(R_a)_2)-$ or $-C(R_b)_2-$;

X is $-O-$, $-S-$, $-N(R_a)-$, $-C(=O)-$, or $-C(R_b)_2-$;

Z is hydrogen, alkyl, haloalkyl, aryl, aralkyl, $-OR_b$, $-N(R_b)_2$, $-C(=O)R_b$, $-C(=NR_a)R_b$, $-C(=NOH)R_b$, $-C(OR_b)(R_b)_2$, $-C(N(R_a)_2)(R_b)_2$ or $-(CH_2CH_2O)_pR_b$;

R_a is hydrogen, alkyl, aryl or aralkyl;

R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl;

p is 0 to 10 inclusive; and

≡ denotes a single bond or a *trans* double bond.

[0194] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein n is 0.

[0195] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein n is 1.

[0196] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein R^1 is hydrogen or methyl.

[0197] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein R^3 is hydrogen.

[0198] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein R^4 is hydrogen or methyl.

[0199] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein X is -O-.

[0200] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein X is -NH-.

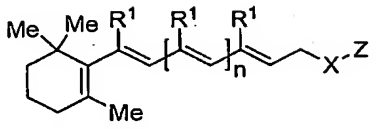
[0201] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein X is $-C(R_b)_2-$.

[0202] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein X is -C(=O)-.

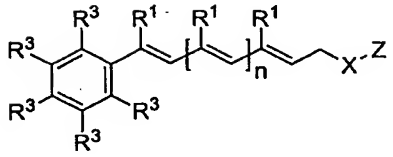
[0203] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein Z is alkyl.

[0204] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIa**, **IIIb**, **IIIc**, or **IIId**, wherein Z is haloalkyl.

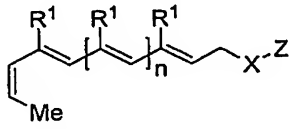
[0205] In certain embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**:



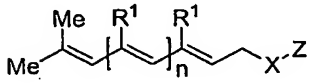
IIIe



IIIf



IIIg



IIIh

wherein, independently for each occurrence,

n is 0 to 4 inclusive;

R^1 is hydrogen or alkyl;

X is -O-, -S-, $-N(R_a)-$, -C(=O)-, or $-C(R_b)_2-$;

Z is hydrogen, alkyl, haloalkyl, aryl, aralkyl, $-OR_b$, $-N(R_b)_2$, $-C(=O)R_b$, $-C(=NR_a)R_b$, $-C(=NOH)R_b$, $-C(OR_b)(R_b)_2$, $-C(N(R_a)_2)(R_b)_2$ or $-(CH_2CH_2O)_pR_b$;

R_a is hydrogen, alkyl, aryl or aralkyl;

R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl; and

p is 0 to 10 inclusive.

[0206] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein n is 0.

[0207] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein n is 1.

[0208] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein R^1 is hydrogen or methyl.

[0209] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein Y is -C(=O)-.

[0210] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein Y is $-CH_2-$.

[0211] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein Z is $-C(=O)R_b$.

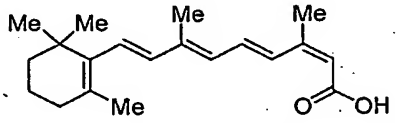
[0212] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein Z is $-CH(OH)R_b-$.

[0213] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein Z is $CH(NH)R_b$.

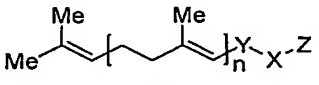
[0214] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein Z is alkyl.

[0215] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IIIe**, **IIIf**, **IIIg**, or **IIIh**, wherein Z is haloalkyl.

[0216] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is 13-*cis*-retinoic acid (isoretinoin, ACCUTANE®):



[0217] In certain embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IV**:



IV

wherein, independently for each occurrence,

n is 1, 2, 3 or 4;

Y is $-C(R_b)_2-$ or $-C(=O)-$;

X is $-O-$, $-NR_a-$, $-C(R_b)_2-$ or $-C(=O)-$;

Z is $-C(=O)R_b$, $-OR_b$, $-N(R_b)_2$, alkyl or haloalkyl;

R_a is hydrogen, alkyl, haloalkyl, aryl or aralkyl; and

R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl.

[0218] In further embodiments, an inhibitor of retinal pigment epithelium (RPE65) has a structure represented by formula **IV**, wherein Y is $-CH_2-$.

[0219] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IV**, wherein X is -O-.

[0220] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IV**, wherein Z is $-C(=O)R_b$; and R_b is alkyl.

[0221] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IV**, wherein Z is alkyl.

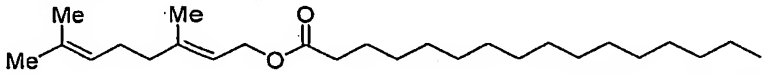
[0222] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IV**, wherein Y is $-CH_2-$; X is -O-; Z is $-C(=O)R_b$; and R_b is alkyl.

[0223] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula **IV**, wherein Y is $-CH_2-$; X is -O-; and Z is alkyl.

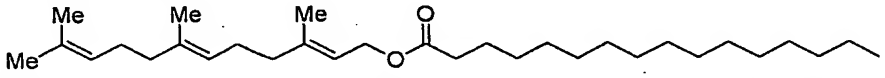
[0224] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula IV, wherein Y is -CH_2-; X is -C(=O)-; and Z is alkyl.

[0225] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula IV, wherein Y is -CH_2-; X is -C(=O)-; Z is -$N(R_b)_2$; and R_b is alkyl.

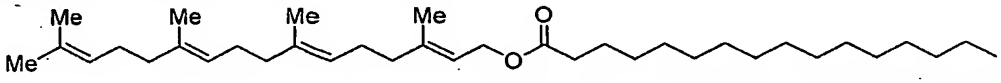
[0226] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is geranyl palmitate (K_D = 301 nM):



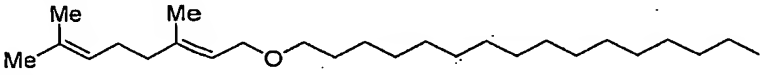
[0227] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is farnesyl palmitate (K_D = 63 nM)



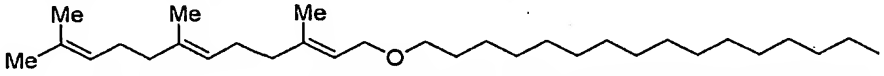
[0228] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is geranylgeranyl palmitate (K_D = 213 nM):



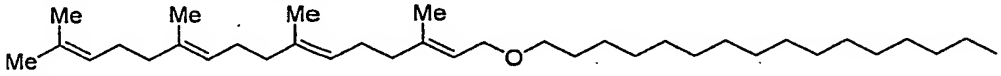
[0229] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is geranyl palmityl ether (K_D = 416 nM):



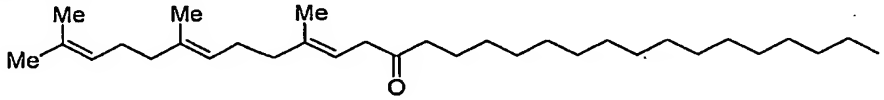
[0230] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is farnesyl palmityl ether (K_D = 60 nM):



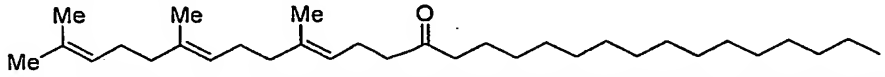
[0231] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is geranylgeranyl palmityl ether (K_D = 195 nM):



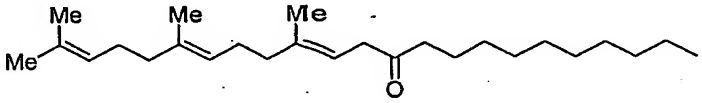
[0232] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound:



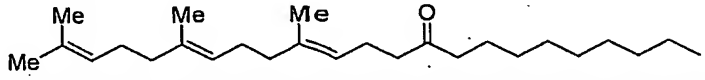
[0233] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound (K_D = 96 nM):



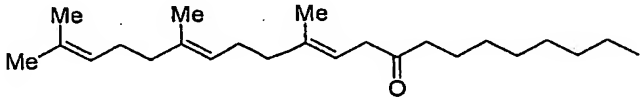
[0234] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound:



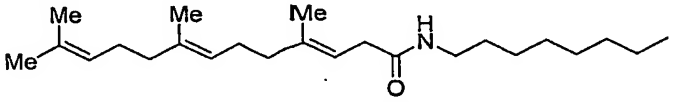
[0235] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound (K_D = 56 nM):



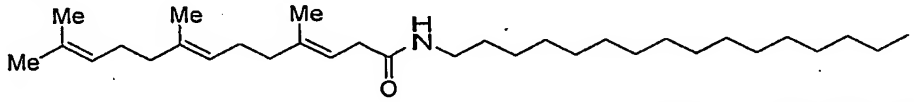
[0236] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is farnesyl octyl ketone:



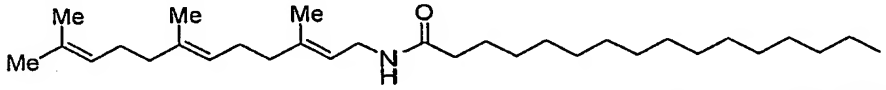
[0237] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is octyl farnesimide:



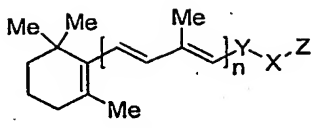
[0238] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is palmityl farnesimide:



[0239] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound (K_D = 56 nM):



[0240] In certain embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V:



V

wherein, independently for each occurrence,

n is 1, 2 or 3;

Y is $-C(R_b)_2-$, -C(=O)- or -CH(OH)-;

X is -O-, $-NR_a-$ or $-C(R_b)_2-$;

Z is $-C(=O)R_b$, hydrogen, $-(CH_2CH_2O)_pR_b$, alkyl or haloalkyl;

R_a is hydrogen, alkyl, haloalkyl, aryl or aralkyl;

R_b is hydrogen, alkyl, haloalkyl, aryl or aralkyl; and

p is 1 to 10 inclusive.

[0241] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein Y is $-CH_2-$.

[0242] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein Y is -C(=O)-.

[0243] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein Y is -CH(OH)-.

[0244] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein X is -O-.

[0245] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein X is $-NR_a-$

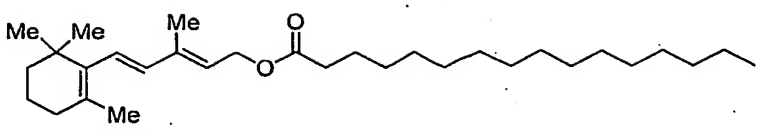
[0246] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein X is $-C(R_b)-$.

[0247] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein Z is alkyl.

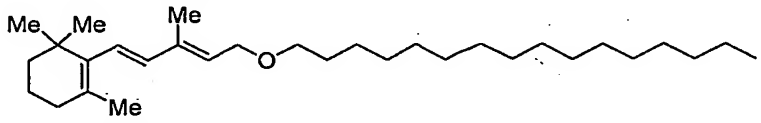
[0248] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein Z is $-C(=O)R_b$; and R_b is alkyl.

[0249] In further embodiments, an antagonist of retinal pigment epithelium (RPE65) has a structure represented by formula V, wherein Z is $-(CH_2CH_2O)_pR_b$; and R_b is alkyl.

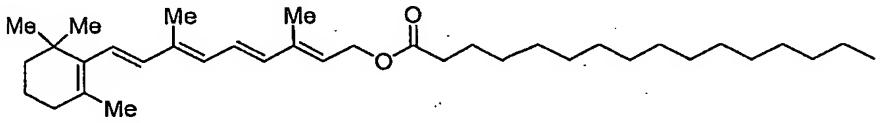
[0250] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is β-ionoacetyl palmitate (K_D = 153 nM):



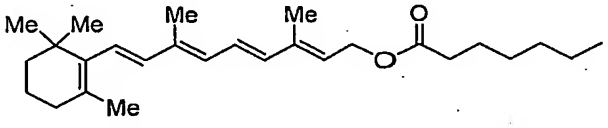
[0251] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is β-ionoacetyl palmityl ether (K_D = 156 nM):



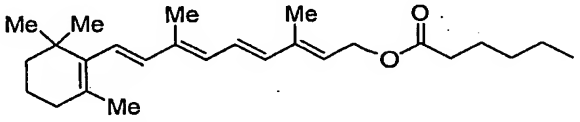
[0252] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is retinyl palmitate (**4a**; K_D = 47 nM):



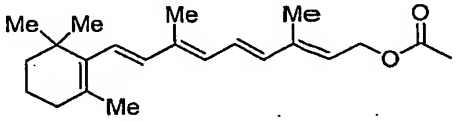
[0253] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is retinyl hexanoate (**4b**; K_D = 235 nM):



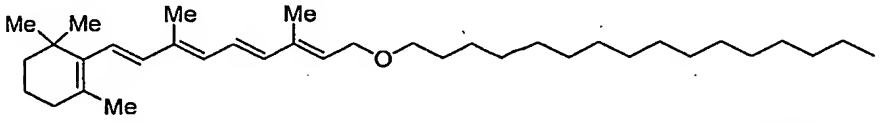
[0254] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is retinyl pentanoate:



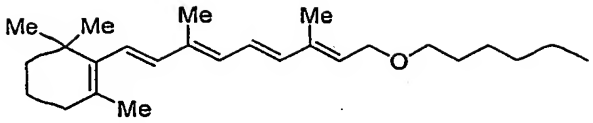
[0255] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is retinyl acetate (**4c**; K_D = 1,300 nM):



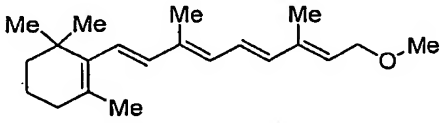
[0256] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is palmityl retinyl ether (**4d**, K_D = 25 nM):



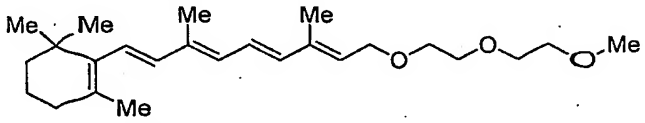
[0257] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is hexyl retinyl ether (K_D = 151 nM):



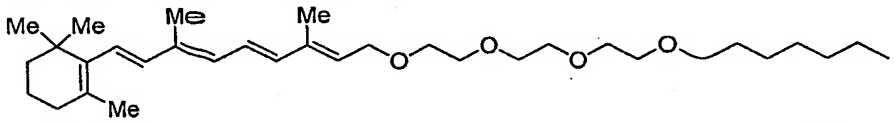
[0258] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is methyl retinyl ether (K_D = 24 nM):



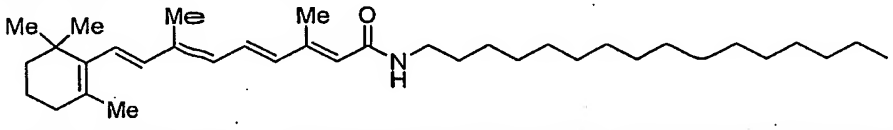
[0259] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is retinyl [2-(2'-methoxy)ethoxy]ethyl ether (K_D = 486 nM):



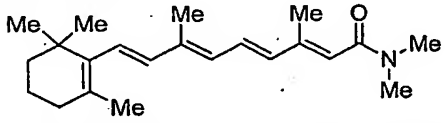
[0260] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is:



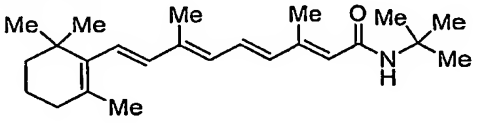
[0261] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is *N*-palmityl retinimide (K_D = 40 nM):



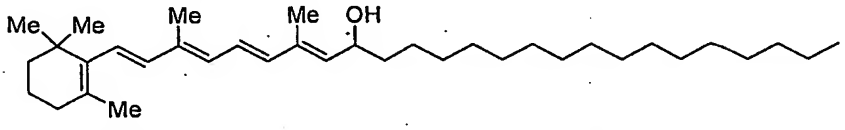
[0262] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is *N,N*-dimethyl retinimide (K_D = 3,577 nM):



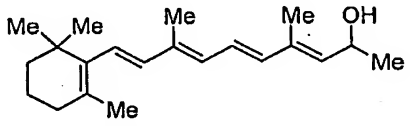
[0263] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is *N-tert*-butyl retinimide (K_D = 4,321 nM):



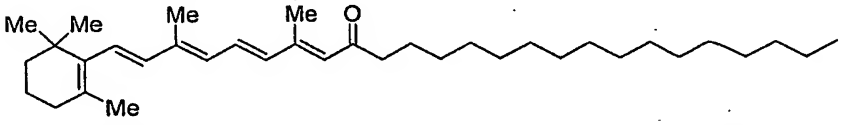
[0264] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is palmityl retinyl alcohol (K_D = 170 nM):



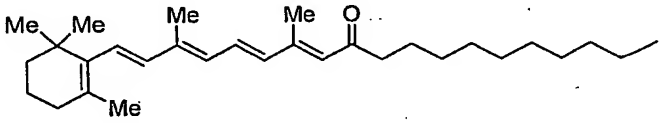
[0265] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is methyl retinyl alcohol:



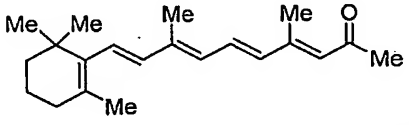
[0266] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is palmityl retinyl ketone (K_D = 64 nM):



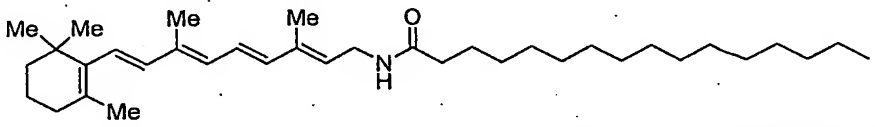
[0267] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is retinyl decyl ketone:



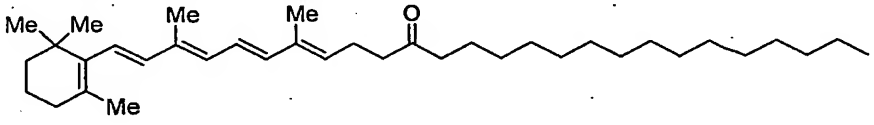
[0268] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is methyl retinyl ketone (K_D = 3,786 nM):



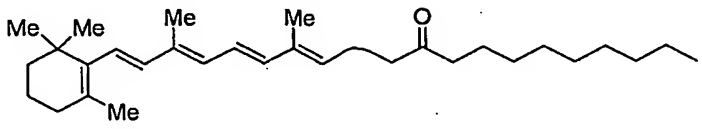
[0269] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound (4e):



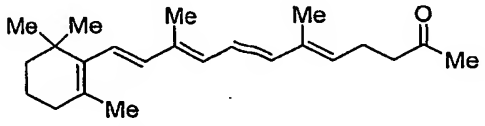
[0270] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound (4f; K_D = 64 nM)



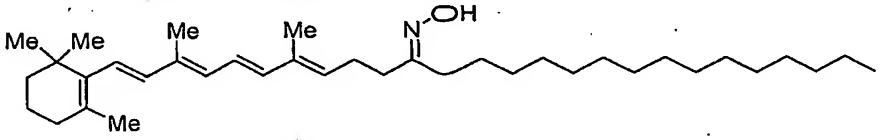
[0271] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound (K_D = 173 nM):



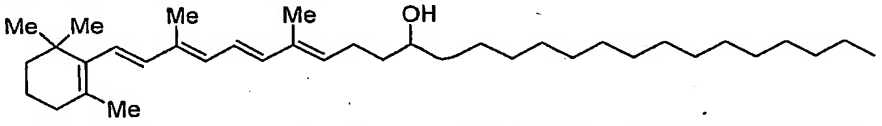
[0272] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is the following compound (K_D = 3,786 nM):



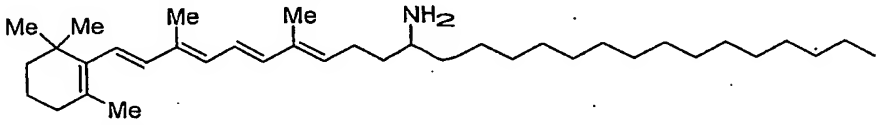
[0273] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is:



[0274] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is:



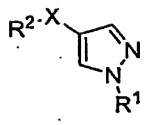
[0275] In one embodiment, an antagonist of retinal pigment epithelium (RPE65) is:



[0276] The above-described RPE65 antagonist compounds and general formulas of compounds, with their various substituent definitions and further embodiments, are also LRAT inhibitors, and are incorporated herein by reference as LRAT inhibitors.

[0277] Other antagonists of RPE65 and inhibitors of LRAT include agents that inhibit palmitoylation. For example, 2-bromopalmitate inhibits palmitoylation. In some embodiments, a racemic mixture of 2-bromopalmitate may be applied to inhibit LRAT and/or antagonize RPE65. In other embodiments, purified (*R*)-2-bromopalmitic acid may be applied to inhibit LRAT and/or antagonize RPE65. In yet other embodiments, purified (*S*)-2-bromopalmitic acid may be applied to inhibit LRAT and/or antagonize RPE65.

[0278] In certain embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula VI:



VI

wherein, independently for each occurrence,

R^1 is hydrogen, alkyl, aryl or aralkyl;

X is alkyl, alkenyl, $-C(R_b)_2-$, $-C(=O)-$, $-C(=NR_a)-$, $-C(OH)R_b$ or $-C(N(R_a)_2)R_b-$;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

R_a is hydrogen, alkyl, aryl or aralkyl; and

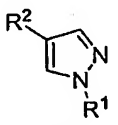
R_b is hydrogen or alkyl.

[0279] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VI**, wherein R^1 is hydrogen.

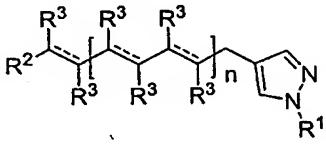
[0280] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VI**, wherein X is $-C(R_b)_2-$.

[0281] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VI**, wherein X is $-C(=O)-$.

[0282] In certain embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIa** or **VIb**:



VIa



VIb

wherein, independently for each occurrence,

R^1 is hydrogen, alkyl, aryl or aralkyl;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

R^3 is hydrogen or alkyl;

R_a is hydrogen, alkyl, aryl or aralkyl;

R_b is hydrogen or alkyl; and

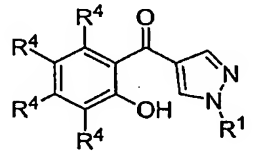
=== denotes a single bond, a *cis* double bond, or a *trans* double bond.

[0283] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIa** or **VIb**, wherein R^1 is hydrogen.

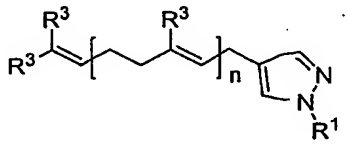
[0284] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIa** or **VIb**, wherein R^2 is alkyl.

[0285] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIa** or **VIb**, wherein R^3 is hydrogen or methyl.

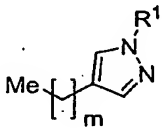
[0286] In certain embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIc**, **VId** or **VIe**:



VIc



VId



VIe

wherein, independently for each occurrence,

n is 1 to 5 inclusive;

m is 0 to 30 inclusive;

R^1 is hydrogen, alkyl, aryl or aralkyl;

R^2 is hydrogen, alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, or aralkyl;

R^3 is hydrogen or alkyl;

R^4 is hydrogen, halogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, aralkyenyl, aralkynyl, heteroaralkyl, heteroaralkyenyl, heteroaralkynyl, cyano, nitro, sulfhydryl, hydroxyl, sulfonyl, amino, acylamino, amido, alkylthio, carboxyl, carbamoyl, alkoxyl, sulfonate, sulfate, sulfonamido, sulfamoyl, sulfonyl, and sulfoxido;

R_a is hydrogen, alkyl, aryl or aralkyl; and

R_b is hydrogen or alkyl.

[0287] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIc**, wherein R^1 is hydrogen.

[0288] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIc**, wherein R^4 is hydrogen.

[0289] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIc**, wherein R^1 is hydrogen; and R^4 is hydrogen.

[0290] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VId**, wherein n is 1, 2 or 3.

[0291] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VId**, wherein R^3 is methyl.

[0292] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VId**, wherein R^1 is hydrogen.

[0293] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VId**, wherein n is 1, 2 or 3; R^3 is methyl.

[0294] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VId**, wherein n is 1, 2 or 3; R^3 is methyl; and R^1 is hydrogen.

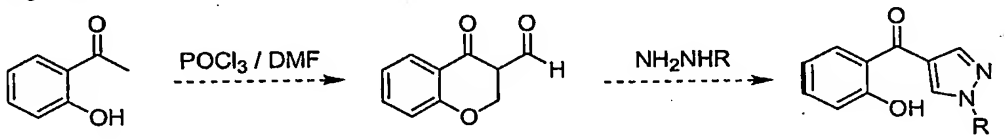
[0295] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIe**, wherein R^1 is hydrogen.

[0296] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIe**, wherein m is 1 to 10 inclusive.

[0297] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIe**, wherein m is 11 to 20 inclusive.

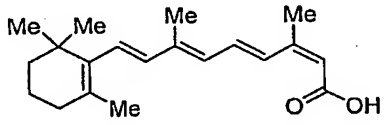
[0298] In further embodiments, an inhibitor of 11-*cis*-retinol dehydrogenase has a structure represented by formula **VIe**, wherein m is 11 to 20 inclusive; and R^1 is hydrogen.

[0299] 11-*cis*-retinol dehydrogenase inhibitors having structures represented by formular **VIe** may be generated according to a diversity library approach as shown in Scheme 1, among other ways:



Scheme 1

[0300] In one embodiment, an inhibitor of 11-*cis*-retinol dehydrogenas is 13-*cis*-retinoic acid (isoretinoin, ACCUTANE®):

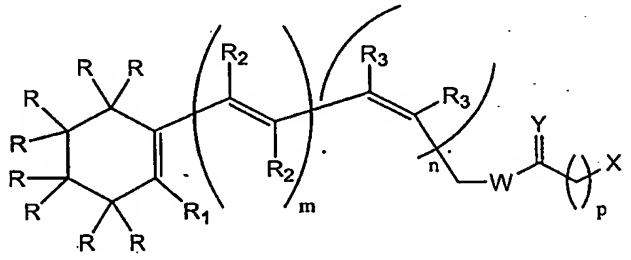


[0301] Also included are pharmaceutically acceptable addition salts and complexes of the compounds of the formulas given above. In cases wherein the compounds may have one or more chiral centers, unless specified, the compounds contemplated herein may be a single stereoisomer or racemic mixtures of stereoisomers. Further included are prodrugs, analogs, and derivatives thereof.

[0302] In some embodiments, two or more enzyme inhibitors and/or RPE65 binding inhibitors may be combined. In some embodiments, an enzyme inhibitor and/or RPE65 binding inhibitor may be combined with a short-circuiting compound. Combinations may be selected to inhibit sequential steps in the visual cycle (that is, two steps that occur one

immediately after the other).

[0303] In certain embodiments, an inhibitor of isomerohydrolase (IMH) may be a compound having a structure represented by general structure 1:



1

wherein, independently for each occurrence:

R, R_1, R_2, and R_3 are H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

W and Y are O, NR, R, or S;

X is H, alkyl, haloalkyl, aryl, or halide;

m and n are integers from 1 to 6 inclusive; and

p is an integer from 0 to 6 inclusive.

[0304] In a further embodiment, the inhibitor of IMH has the structure of formula 1 and the attendant definitions, wherein R_2 and R_3 is H or Me.

[0305] In a further embodiment, the inhibitor of IMH has the structure of formula 1 and the attendant definitions, wherein m is 2.

[0306] In a further embodiment, the inhibitor of IMH has the structure of formula 1 and the attendant definitions, wherein n is 2.

[0307] In a further embodiment, the inhibitor of IMH has the structure of formula 1 and the attendant definitions, wherein W is O.

[0308] In a further embodiment, the inhibitor of IMH has the structure of formula 1 and the attendant definitions, wherein W is C.

[0309] In a further embodiment, the inhibitor of IMH has the structure of formula 1 and the attendant definitions, wherein Y is O.

[0310] In a further embodiment, the inhibitor of IMH has the structure of formula 1 and the attendant definitions, wherein p is 1.

[0311] In a further embodiment, the inhibitor of IMH has the structure of formula 1 and the attendant definitions, wherein X is Br.

[0312] In a further embodiment, the inhibitor of IMH has the structure of formula **1** and the attendant definitions, wherein R_2 and R_3 is H or Me, and m is 2.

[0313] In a further embodiment, the inhibitor of IMH has the structure of formula **1** and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 2, and n is 2.

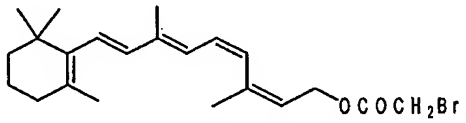
[0314] In a further embodiment, the inhibitor of IMH has the structure of formula **1** and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 2, n is 2, and W is O.

[0315] In a further embodiment, the inhibitor of IMH has the structure of formula **1** and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 2, n is 2, W is O, and Y is O.

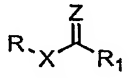
[0316] In a further embodiment, the inhibitor of IMH has the structure of formula **1** and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 2, n is 2, W is O, Y is O, and p is 1.

[0317] In a further embodiment, the inhibitor of IMH has the structure of formula **1** and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 2, n is 2, W is O, Y is O, p is 1, and X is Br.

[0318] In one embodiment, an isomerohydrolase inhibitor is 11-*cis*-retinyl bromoacetate (cRBA):



[0319] In certain embodiments, an inhibitor of IMH may be a compound of formula **8a**:



8a

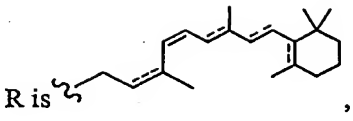
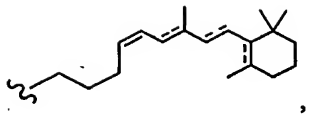
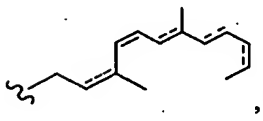
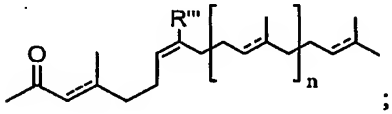
wherein, independently for each occurrence:

X is O, S, NR′, CH_2, or NHNR′;

Z is O or NOH;

R_1 is $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2N_2$, $-CH_2C(O)OR$, $-OR'$, $-C(O)CHR'$, $-C(NH)CHR'$, or $-CH=CHR'$;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  ,  ,  , or  ;

R‴ is CH_3 or H; and

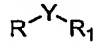
n is 0, 1 or 2;

wherein === denotes a single bond, a *cis* double bond or a *trans* double bond.

[0320] Compounds of formula **8a** may be considered irreversible inhibitors of IMH because they can covalently bind IMH, permanently disabling it.

[0321] In certain embodiments, an inhibitor of IMH may be a compound of formula **8a** wherein Z is O.

[0322] In certain embodiments, an inhibitor of IMH may be a compound of formula **8b**:



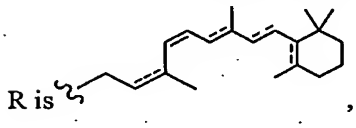
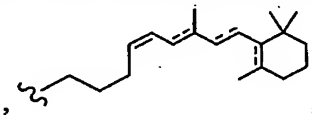
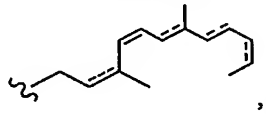
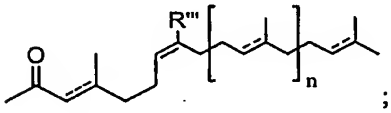
8b

wherein, independently for each occurrence:

Y is C=O, C=S, C=NR′, or CH_2;

R_1 is R′, -OR′, or -CN;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  ,  ,  , or  ;

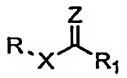
R‴ is CH_3 or H; and

n is 0, 1, or 2;

wherein === denotes a single bond, a *cis* double bond or a *trans* double bond.

[0323] Compounds of formula **8b** may be considered reversible inhibitors of IMH because they can noncovalently bind IMH without permanently disabling it.

[0324] In certain embodiments, an inhibitor of IMH may be a compound of formula **8c**:



8c

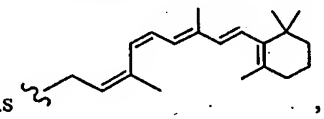
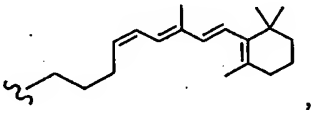
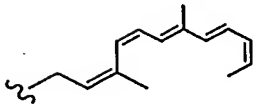
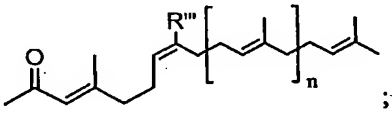
wherein, independently for each occurrence:

X is O, S, NR′, CH_2, or NHNR′;

Z is O or NOH;

R_1 is $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2N_2$, $-CH_2C(O)OR$, -OR′, -C(O)CHR′, -C(NH)CHR′, or -CH=CHR′;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  ,  ,  , or  ;

R′′′ is CH_3 or H; and

n is 0, 1 or 2.

[0325] Compounds of formula **8c** may be considered irreversible inhibitors of IMH because they can covalently bind IMH, permanently disabling it.

[0326] In certain embodiments, an inhibitor of IMH may be a compound of formula **8c** wherein Z is O.

[0327] In certain embodiments, an inhibitor of IMH may be a compound of formula **8d**:

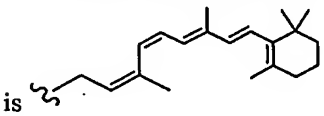
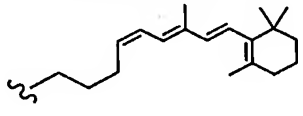
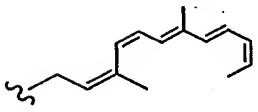
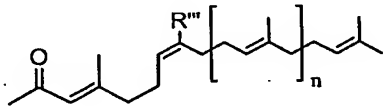
8d

wherein, independently for each occurrence:

Y is C=O, C=S, C=NR′, or CH_2;

R_1 is R′, -OR′, or -CN;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

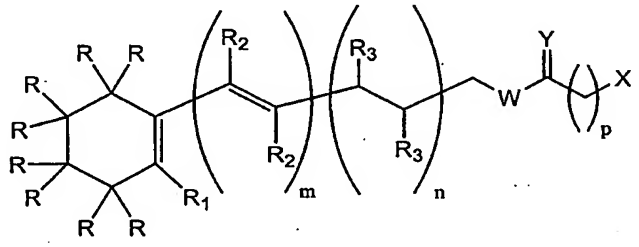
R is  ,  ,  , or  ;

R′′′ is CH_3 or H; and

n is 0, 1 or 2.

[0328] Compounds of formula **8d** may be considered reversible inhibitors of IMH because they can noncovalently bind IMH without permanently disabling it.

[0329] In certain embodiments, an inhibitor of LRAT may be a compound having a structure represented by general structure **2**:



2

wherein, independently for each occurrence:

R, R_1, R_2, and R_3 are H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

W and Y are O, NR, R, or S;

X is H, alkyl, haloalkyl, or aryl;

m and n are integers from 1 to 6 inclusive; and

p is an integer from 0 to 6 inclusive.

[0330] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein R_2 and R_3 is H or Me.

[0331] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein m is 3.

[0332] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein n is 1.

[0333] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein W is O.

[0334] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein W is C.

[0335] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein Y is O.

[0336] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein p is 0.

[0337] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein X is OCF_3.

[0338] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein R_2 and R_3 is H or Me, and m is 3.

[0339] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 3, and n is 1.

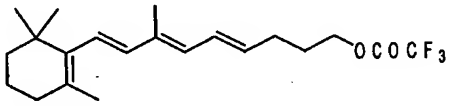
[0340] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 3, n is 1, and W is O.

[0341] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 3, n is 1, W is O, and Y is O.

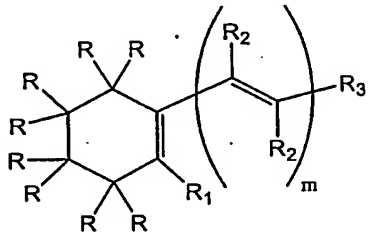
[0342] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 3, n is 1, W is O, Y is O, and p is 0.

[0343] In a further embodiment, the inhibitor of LRAT has the structure of formula 2 and the attendant definitions, wherein R_2 and R_3 is H or Me, m is 3, n is 1, W is O, Y is O, p is 0, and X is OCF_3.

[0344] An exemplary inhibitor of LRAT is all-*trans*-retinyl α-bromoacetate. Another exemplary inhibitor of LRAT is 13-desmethyl-13,14-dihydro-all-*trans*-retinyl trifluoroacetate (RFA):



[0345] In certain embodiments, a compound that interferes with RPE65 binding may be a compound having a structure represented by general structure 3:



3

wherein, independently for each occurrence:

R and R_1 are H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, or heteroaralkyl;

R_2 is H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or -CO_2R;

R_3 is H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or -CH_2OR_4;

R_4 is H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, heterocyclyl; and

m is an integer from 1 to 6 inclusive.

[0346] In a further embodiment, the inhibitor of LRAT has the structure of formula 3 and the attendant definitions, wherein R_2 is H, Me, or -CO_2H.

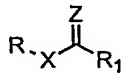
[0347] In a further embodiment, the inhibitor of LRAT has the structure of formula 3 and the attendant definitions, wherein m is 4.

[0348] In a further embodiment, the inhibitor of LRAT has the structure of formula 3 and the attendant definitions, wherein R_3 is H.

[0349] In a further embodiment, the inhibitor of LRAT has the structure of formula 3 and the attendant definitions, wherein R_2 is H, Me, or -CO_2H and m is 4.

[0350] In a further embodiment, the inhibitor of LRAT has the structure of formula 3 and the attendant definitions, wherein R_2 is H, Me, or -CO_2H, m is 4, and R_3 is H.

[0351] In certain embodiments, an inhibitor of LRAT may be a compound of formula 6a:



6a

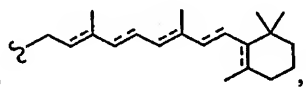
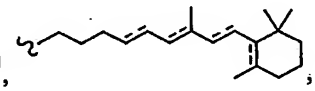
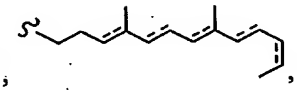
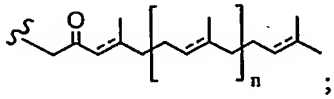
wherein, independently for each occurrence:

X is O, S, NR′, CH_2, or NHNR′;

Z is O or NOH;

R_1 is -CH_2F, -CHF_2, -CF_3, -CH_2N_2, -$CH_2C(O)OR$, -OR′, -C(O)CHR′, -C(NH)CHR′, or -CH=CHR′;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  ,  ,  , or  ; and

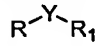
n is 1, 2, or 3;

wherein ≡≡≡ denotes a single bond, a *cis* double bond or a *trans* double bond.

[0352] Compounds of formula 6a may be considered irreversible inhibitors of LRAT because they can covalently bind LRAT, permanently disabling it.

[0353] In certain embodiments, an inhibitor of LRAT may be a compound of formula 6a wherein Z is O.

[0354] In certain embodiments, an inhibitor of LRAT may be a compound of formula 6b:



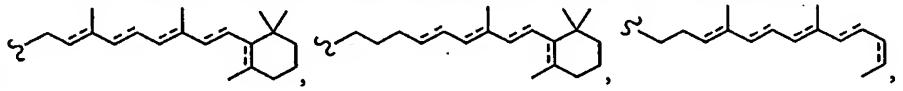
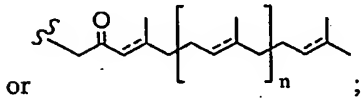
6b

wherein, independently for each occurrence:

Y is C=O, C=S, C=NR′, or CH_2;

R_1 is R′, -OR′, or -CN;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  , 

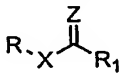
or ; and

n is 1, 2, or 3;

wherein ≡≡≡ denotes a single bond, a *cis* double bond or a *trans* double bond.

[0355] Compounds of formula 6c may be considered reversible inhibitors of LRAT because they can noncovalently bind LRAT without permanently disabling it.

[0356] In certain embodiments, an inhibitor of LRAT may be a compound of formula 6c:



6c

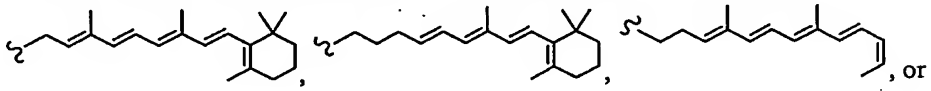
wherein independently for each occurrence:

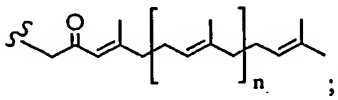
X is O, S, NR′, CH_2, or NHNR′;

Z is O or NOH;

R_1 is $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2N_2$, $-CH_2C(O)OR$, -OR′, -C(O)CHR′, -C(NH)CHR′, or -CH=CHR′;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  , or

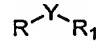
 ; and

n is 1, 2, or 3.

[0357] Compounds of formula **6c** may be considered irreversible inhibitors of LRAT because they can covalently bind LRAT, permanently disabling it.

[0358] In certain embodiments, an inhibitor of LRAT may be a compound of formula **6c** wherein Z is O.

[0359] In certain embodiments, an inhibitor of LRAT may be a compound of formula **6d**:



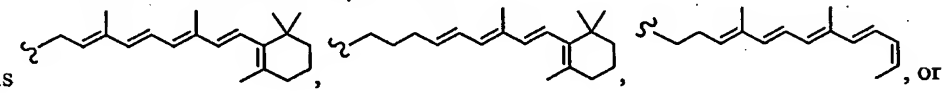
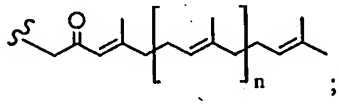
6d

wherein, independently for each occurrence:

Y is C=O, C=S, C=NR′, or CH_2;

R_1 is R′, -OR′, or -CN;

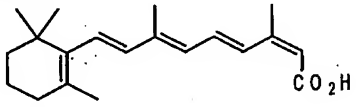
R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  , or  ; and

n is 1, 2, or 3.

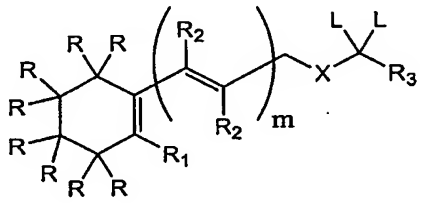
[0360] Compounds of formula **6d** may be considered reversible inhibitors of LRAT because they can noncovalently bind LRAT without permanently disabling it.

[0361] One exemplary embodiment of a compound that interferes with RPE65 binding is 13-*cis*-retinoic acid (isotretinoin, ACCUTANE®):



[0362] 13-*cis*-retinoic acid is converted *in vivo* to all-*trans*-retinoic acid, which is a powerful inhibitor of RPE65 function.

[0363] In certain embodiments, an antagonist of RPE65 is a compound having a structure represented by general structure **4**:



4

wherein, independently for each occurrence:

R, R_1, R_2 are H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, alkoxy, aryloxy, amino, halo, hydroxy, or carboxyl;

R_3 is alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or ether;

L is H, OH, NH_2, $N(R)_2$, alkoxy, aryloxy, halo, hydroxy, carboxyl, or two L taken together represent O, S, or NR;

X is $C(R)_2$, O, S, or NR; and

m is an integer from 1 to 6 inclusive.

[0364] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O.

[0365] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is CH_2.

[0366] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is NH.

[0367] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein two Ls taken together represent O.

[0368] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein two Ls taken together represent NOH.

[0369] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein L is H, OH, or NH_2.

[0370] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein each L is H.

[0371] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein m is 4.

[0372] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein m is 3.

[0373] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein R_2 is H or methyl.

[0374] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein R_3 is alkyl.

[0375] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein R_3 is ether.

[0376] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O and two L taken together represents O.

[0377] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O and each L is H.

[0378] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is NH and two L taken together represents O.

[0379] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is CH_2 and two L taken together represents O.

[0380] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is CH_2 and two L taken together represents NOH.

[0381] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O, two L taken together represent O, R_2 is H or methyl, m is 4, and R_3 is a C15 alkyl.

[0382] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O, two L taken together represent O, R_2 is H or methyl, m is 4, and R_3 is a C5 alkyl.

[0383] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O, two L taken together represent O, R_2 is H or methyl, m is 4, and R_3 is methyl.

[0384] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O, each L is H, R_2 is H or methyl, m is 4, and R_3 is a C15 alkyl.

[0385] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is NH, two L taken together represents O, R_2 is H or methyl, m is 4, and R_3 is a C15 alkyl.

[0386] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is CH_2, two L taken together represents O, R_2 is H or methyl, m is 4, and R_3 is a C15 alkyl.

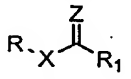
[0387] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O, each L is H, R_2 is H or methyl, m is 4, and R_3 is an ether.

[0388] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is O, each L is H, R_2 is H or methyl, m is 4, and R_3 is -$CH_2OCH_2CH_2OCH_2CH_2OC_7H_{15}$.

[0389] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is CH_2, two L taken together represent NOH, R_2 is H or methyl, m is 4, and R_3 is a C15 alkyl.

[0390] In a further embodiment, an RPE65 antagonist has the structure of formula 4 and the attendant definitions, wherein X is CH_2, L is H, OH, or NH_2, R_2 is H or methyl, m is 4, and R_3 is a C15 alkyl.

[0391] In certain embodiments, an inhibitor of RPE65 may be a compound of formula **7a**:



7a

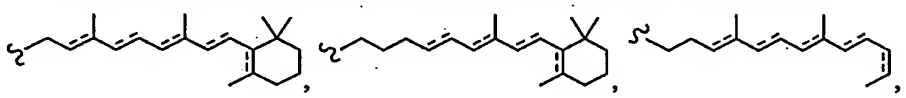
wherein, independently for each occurrence:

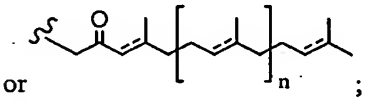
X is O, S, NR′, CH_2, or NHNR′;

Z is O or NOH;

R_1 is -CH_2F, -CHF_2, -CF_3, -CH_2N_2, -$CH_2C(O)OR$, -OR′, -C(O)CHR′, -C(NH)CHR′, or -CH=CHR′;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  ,

or  ; and

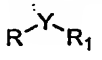
n is 1, 2, or 3;

wherein ≡ denotes a single bond, a *cis* double bond or a *trans* double bond.

[0392] Compounds of formula **7a** may be considered irreversible antagonists of RPE65 because they can covalently bind RPE65, permanently disabling it.

[0393] In certain embodiments, an inhibitor of RPE65 may be a compound of formula **7a** wherein Z is O.

[0394] In certain embodiments, an inhibitor of RPE65 may be a compound of formula **7b**:



7b

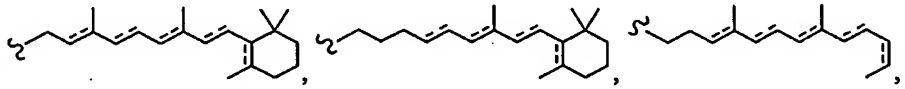
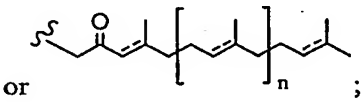
wherein, independently for each occurrence:

Y is O, S, NR′, CH_2=O, C=S, C=NR′, CHOR', CHNR′R′′, CHSR′, or CH_2;

R_1 is R′, -OR′, -CN or $(CH_2CH_2O)_mR'$;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R′′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  ,  ;

or

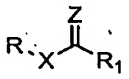
m is 1, 2 or 3; and

n is 1, 2, or 3;

wherein ≡ denotes a single bond, a *cis* double bond or a *trans* double bond.

[0395] Compounds of formula **7b** may be considered reversible antagnoists of RPE65 because they can noncovalently bind RPE65 without permanently disabling it.

[0396] In certain embodiments, an inhibitor of RPE65 may be a compound of formula **7c**:



7c

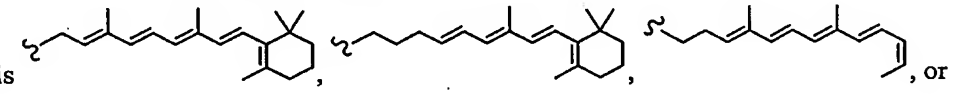
wherein, independently for each occurrence:

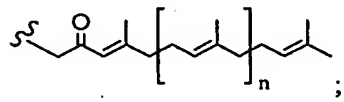
X is O, S, NR′, CH_2, or NHNR′;

Z is O or NOH;

R_1 is $-CH_2F$, $-CHF_2$, $-CF_3$, $-CH_2N_2$, $-CH_2C(O)OR$, -OR′, -C(O)CHR′, -C(NH)CHR′, or -CH=CHR′;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is  , or

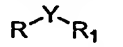
 ; and

n is 1, 2, or 3.

[0397] Compounds of formula **7c** may be considered irreversible antagonists of RPE65 because they can covalently bind RPE65, permanently disabling it.

[0398] In certain embodiments, an inhibitor of RPE65 may be a compound of formula **7c** wherein Z is O.

[0399] In certain embodiments, an inhibitor of RPE65 may be a compound of formula **7d**:



7d

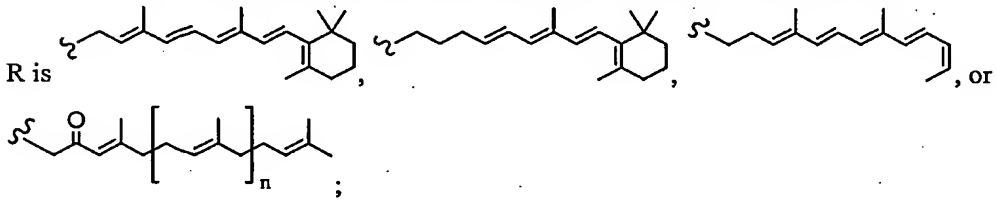
wherein, independently for each occurrence:

Y is C=O, C=S, C=NR′, CHOH, CHOR′, NH_2, NHR′, NR′R″, SH, SR′, or CH_2;

R_1 is R′, -OR′, -CN or $-(CH_2CH_2O)_mR'$;

R′ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R″ is H, alkyl, heteroalkyl, aryl, heteroaryl, aralkyl, or heteroaralkyl;

R is 

m is 1, 2 or 3; and

n is 1, 2, or 3.

[0400] B. Compositions for short-circuiting

[0401] Short-circuiting the visual cycle can be achieved by catalyzing the thermodynamically downhill isomerization of 11-*cis*-retinal to all-*trans*-retinal in the RPE, before the 11-*cis*-retinal leaves the RPE. Figure 3 depicts one contemplated intervention. A very wide variety of substances are envisioned as appropriate for this use. Broadly speaking, appropriate drugs include aniline derivates, i.e., a benzene ring with an amine side chain.

[0402] Short circuiting molecules operate by first forming a Schiff base with a retinal. When a Schiff base is formed with 11-*cis*-retinal, isomerization occurs. This is the short circuit.

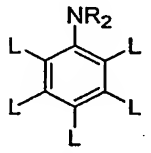
[0403] Short-circuit compounds may also trap retinals so that they are not available to form A_2E, its precursors or analogs. With all-*trans*-retinal, a relatively stable Schiff base can be formed with the drugs which traps the all-*trans*-retinal and prevents it from forming A_2E and like compounds. The short-circuit drug competes with phosphatidylethanolamine for binding all-*trans*-retinal. The trapped compounds may then be broken down in lysozomes to non-toxic metabolites. A short-circuit drug may disrupt the visual cycle in one or both ways, i.e., by short-circuiting 11-*cis*-retinals and/or by trapping all-*trans*-retinals. (A_2E is the best characterized of the lipofuscins. There may be other adducts between all-*trans*-

retinal and amines -- or even proteins -- whose formation is initiated by Schiff base formation between a reactive retinal and an amine.)

[0404] While it is not expected that an aromatic amine/all-*trans*-retinal Schiff base will go on to form A_2E-like molecules (because it will be degraded first), this can be more reliably prevented by using a short-circuiting drug that is a secondary amine. This is because the mechanism of A_2E formation requires a primary amine (two free Hs) because two new N-alkyl bonds are made (one with each all-*trans*-retinal molecule) and this cannot happen starting with a secondary or tertiary amine. If the short-circuit drug is a secondary amine, then it can bind only one molecule of all-*trans*-retinal and has no remaining site to bind a second all-*trans*-retinal, thereby preventing the formation of compounds analogous to A_2E akin to the process shown in Figure 2.

[0405] Short-circuit drugs may also provide a long-term effect, so that their administration can be infrequent. In some cases, administration may be required monthly. In other cases, administration may be required weekly. The short-circuit drugs effectively deplete vitamin A stores locally in the eye by trapping all-*trans*-retinal. Once the store of vitamin is diminished by the drug, the visual cycle is impaired, and lipofuscin formation is retarded, which is the goal of therapy. Vitamin A stores are replenished only very slowly in the eye, so that a single administration of short-circuit drug may have a prolonged effect. In addition, the short-circuit drugs may be cleared slowly from the eye, so that they may be available for binding over extended periods.

[0406] In certain embodiments, a short-circuiting compound has the structure represented by formula **VII**:



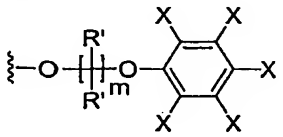
VII

wherein, independently for each occurrence:

R is H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or carbonyl;

L is a hydrophobic moiety, or any two adjacent L taken together form a fused aromatic or heteroaromatic ring (*e.g.* a naphthalene, an anthracene, an indole, a quinoline, *etc.*).

[0407] In certain embodiments, independently for each occurrence, L is alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, carbonyl, ether, or polycyclic. In certain embodiments, L has the formula **VIIa**:



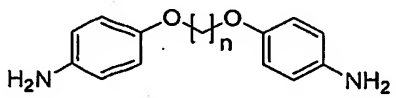
VIIa

wherein, independently for each occurrence:

R' and X are hydrogen, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, carbonyl, alkoxy, hydroxy, thiol, thioalkyl, or amino; and

m is an integer from 1 to 6 inclusive.

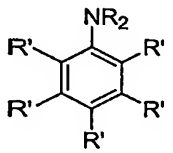
[0408] In some embodiments, a short circuit drug may be represented by the following generic formula **VIIb**:



VIIb

[0409] wherein n is an integer from 1 to 8 inclusive.

[0410] In some embodiments, a short circuit drug may be represented by the following generic formula **VIIc**:



VIIc

wherein, independently for each occurrence,

R is H, alkyl, or acyl; and

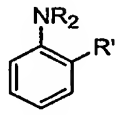
R' is alkyl or ether.

[0411] In a further embodiment, a short circuit drug has the structure of formula **VIIc** and the attendant definitions, wherein R is H for both occurrences.

[0412] In a further embodiment, a short circuit drug has the structure of formula **VIIc** and the attendant definitions, wherein at least one R is alkyl.

[0413] In a further embodiment, a short circuit drug has the structure of formula **VIIc** and the attendant definitions, wherein at least one R is methyl.

[0414] In some embodiments, a short circuit drug may be represented by the following generic formula **VIId**:



VIId

wherein, independently for each occurrence:

R is H, alkyl, or acyl; and

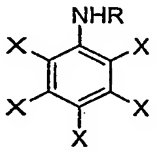
R' is alkyl or ether.

[0415] In a further embodiment, a short circuit drug has the structure of formula **VIId** and the attendant definitions, wherein R is H for both occurrences.

[0416] In a further embodiment, a short circuit drug has the structure of formula **VIId** and the attendant definitions, wherein at least one R is alkyl.

[0417] In a further embodiment, a short circuit drug has the structure of formula **VIId** and the attendant definitions, wherein at least one R is methyl.

[0418] In some embodiments, a short circuit drug may be represented by the following generic formula **VIIe**:



VIIe

wherein, independently for each occurrence:

X is hydrogen or -C(=O)OR';

R is H, alkyl, or acyl; and

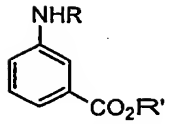
R' is alkyl.

[0419] In a further embodiment, a short circuit drug has the structure of formula **VIIe** and the attendant definitions, wherein R is H.

[0420] In a further embodiment, a short circuit drug has the structure of formula **VIIe** and the attendant definitions, wherein at least one R is alkyl.

[0421] In a further embodiment, a short circuit drug has the structure of formula **VIIe** and the attendant definitions, wherein R is methyl.

[0422] In some embodiments, a short circuit drug may be represented by the following generic formula **VIIf**:



VIIf

wherein, independently for each occurrence

R is H, alkyl, or acyl; and

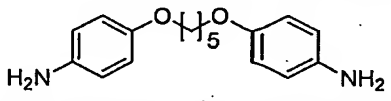
R' is alkyl.

[0423] In a further embodiment, a short circuit drug has the structure of formula VIIf and the attendant definitions, wherein R is H.

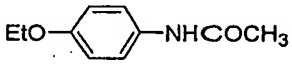
[0424] In a further embodiment, a short circuit drug has the structure of formula VIIf and the attendant definitions, wherein at least one R is alkyl.

[0425] In a further embodiment, a short circuit drug has the structure of formula VIIf and the attendant definitions, wherein R is methyl.

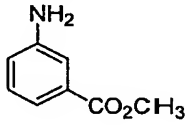
[0426] In one embodiment, a short circuiting drug is diaminophenoxypentane:



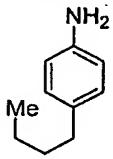
[0427] In one embodiment, a short circuiting drug is phenetidine:



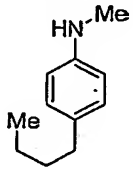
[0428] In one embodiment, a short circuiting drug is and tricaine:



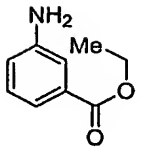
[0429] In one embodiment, a short circuiting drug is 4-butylanaline:



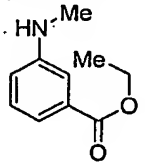
[0430] In one embodiment, a short circuiting drug is *N*-methyl-4-butylanaline:



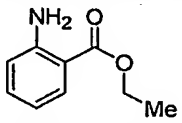
[0431] In one embodiment, a short circuiting drug is ethyl 3-aminobenzoate:



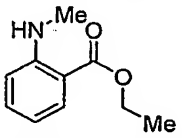
[0432] In one embodiment, a short circuiting drug is ethyl *N*-methyl-3-aminobenzoate:



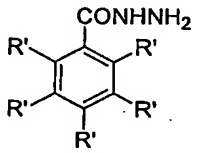
[0433] In one embodiment, a short circuiting drug is ethyl 2-aminobenzoate:



[0434] In one embodiment, a short circuiting drug is ethyl *N*-methyl-2-aminobenzoate:



[0435] In some embodiments, a short circuit drug may be represented by the following generic formula **VIII**:



VIII

wherein R′ is hydrogen, alkyl or ether; or any two adjacent L taken together form a fused aromatic or heteroaromatic ring (*e.g.* a naphthalene, an anthracene, *etc.*).

[0436] In certain embodiments, a short-circuiting compound has the structure represented by formula **IX**:

$$ANR_2$$

IX

wherein, independently for each occurrence:

R is H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or carbonyl; and

A is optionally substituted aryl or heteroaryl.

[0437] In some embodiments, a short circuit drug may be represented by the following generic formula X:

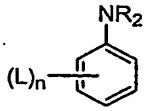
X

wherein independently for each occurrence:

R′ is hydrogen, alkyl or ether; and

A is optionally substituted aryl or heteroaryl.

[0438] In certain embodiments, a short-circuiting compound may have a structure represented by general structure 5:



5

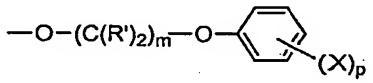
wherein, independently for each occurrence:

R is H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, or carbonyl;

L is a hydrophobic moiety, or any two adjacent L taken together form a fused aromatic ring; and

n is an integer from 0 to 5 inclusive.

[0439] In certain embodiments, independently for each occurrence, L is alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, carbonyl, ether, or polycyclic. In certain embodiments, L has the formula 5a:



5a

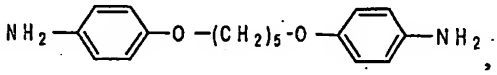
wherein, independently for each occurrence:

R′ and X are H, alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroaralkyl, carbonyl, alkoxy, hydroxy, thiol, thioalkyl, or amino;

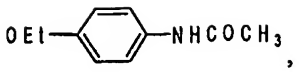
m is an integer from 1 to 6 inclusive; and

p is an integer from 0 to 5 inclusive.

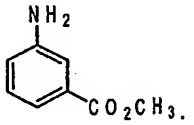
[0440] Selected specific examples of short circuit drugs include diaminophenoxypentane:



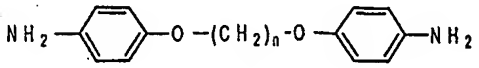
phenetidine:



and tricaine:



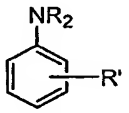
[0441] In some embodiments, a short circuit drug may be represented by the following generic formula **5b**:



5b

wherein n is an integer from 1 to 8 inclusive.

[0442] In some embodiments, a short circuit drug may be represented by the following generic formula **5c**:



5c

wherein, independently for each occurrence:

R is H, alkyl, or acyl; and

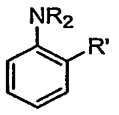
R′ is alkyl or ether.

[0443] In a further embodiment, a short circuit drug has the structure of formula **5c** and the attendant definitions, wherein R is H for both occurrences.

[0444] In a further embodiment, a short circuit drug has the structure of formula **5c** and the attendant definitions, wherein at least one R is alkyl.

[0445] In a further embodiment, a short circuit drug has the structure of formula **5c** and the attendant definitions, wherein at least one R is methyl.

[0446] In some embodiments, a short circuit drug may be represented by the following generic formula **5c1**:



5c1

wherein, independently for each occurrence:

R is H, alkyl, or acyl; and

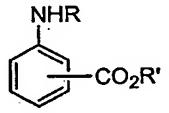
R′ is alkyl or ether.

[0447] In a further embodiment, a short circuit drug has the structure of formula **5c1** and the attendant definitions, wherein R is H for both occurrences.

[0448] In a further embodiment, a short circuit drug has the structure of formula **5c1** and the attendant definitions, wherein at least one R is alkyl.

[0449] In a further embodiment, a short circuit drug has the structure of formula **5c1** and the attendant definitions, wherein at least one R is methyl.

[0450] In some embodiments, a short circuit drug may be represented by the following generic formula **5d:**



5d

wherein, independently for each occurrence:

R is H, alkyl, or acyl; and

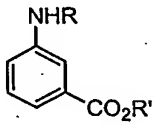
R′ is alkyl.

[0451] In a further embodiment, a short circuit drug has the structure of formula 5d1 and the attendant definitions, wherein R is H.

[0452] In a further embodiment, a short circuit drug has the structure of formula **5d** and the attendant definitions, wherein at least one R is alkyl.

[0453] In a further embodiment, a short circuit drug has the structure of formula **5d** and the attendant definitions, wherein R is methyl.

[0454] In some embodiments, a short circuit drug may be represented by the following generic formula **5d1:**



5d1

wherein, independently for each occurrence:

R is H, alkyl, or acyl; and

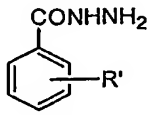
R′ is alkyl.

[0455] In a further embodiment, a short circuit drug has the structure of formula 5d1 and the attendant definitions, wherein R is H.

[0456] In a further embodiment, a short circuit drug has the structure of formula **5d1** and the attendant definitions, wherein at least one R is alkyl.

[0457] In a further embodiment, a short circuit drug has the structure of formula **5d1** and the attendant definitions, wherein R is methyl.

[0458] In some embodiments, a short circuit drug may be represented by the following generic formula **5e**:



5e

wherein R′ is alkyl or ether.

[0459] Also included are pharmaceutically acceptable addition salts and complexes of the compounds of the formulas given above. In cases wherein the compounds may have one or more chiral centers, unless specified, the compounds contemplated herein may be a single stereoisomer or racemic mixtures of stereoisomers. Further included are prodrugs, analogs, and derivatives thereof.

[0460] In some embodiments, two or more short-circuiting compounds may be combined. In some embodiments, an enzyme inhibitor and/or RPE65 binding inhibitor may be combined with a short-circuiting compound.

[0461] Pharmaceutical compositions for use in accordance with the present methods may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Thus, activating compounds and their physiologically acceptable salts and solvates may be formulated for administration by, for example, injection, inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration. In one embodiment, the compound is administered locally, at the site where the target cells, e.g., diseased cells, are present, i.e., in the eye or the retina.

[0462] Compounds can be formulated for a variety of loads of administration, including systemic and topical or localized administration. Techniques and formulations generally may be found in Remmington's Pharmaceutical Sciences, Meade Publishing Co., Easton, PA. For systemic administration, injection is preferred, including intramuscular, intravenous, intraperitoneal, and subcutaneous. For injection, the compounds can be formulated in liquid solutions, preferably in physiologically compatible buffers such as Hank's solution or Ringer's solution. In addition, the compounds may be formulated in

solid form and redissolved or suspended immediately prior to use. Lyophilized forms are also included.

[0463] For oral administration, the pharmaceutical compositions may take the form of, for example, tablets, lozanges, or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., ationd oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

[0464] For administration by inhalation, the compounds may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of e.g., gelatin, for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0465] The compounds may be formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing

and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0466] The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0467] In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0468] Pharmaceutical compositions (including cosmetic preparations) may comprise from about 0.00001 to 100% such as from 0.001 to 10% or from 0.1% to 5% by weight of one or more compounds described herein.

[0469] In one embodiment, a compound described herein, is incorporated into a topical formulation containing a topical carrier that is generally suited to topical drug administration and comprising any such material known in the art. The topical carrier may be selected so as to provide the composition in the desired form, e.g., as an ointment, lotion, cream, microemulsion, gel, oil, solution, or the like, and may be comprised of a material of either naturally occurring or synthetic origin. It is preferable that the selected carrier not adversely affect the active agent or other components of the topical formulation. Examples of suitable topical carriers for use herein include water, alcohols and other nontoxic organic solvents, glycerin, mineral oil, silicone, petroleum jelly, lanolin, fatty acids, vegetable oils, parabens, waxes, and the like.

[0470] Formulations may be colorless, odorless ointments, lotions, creams, microemulsions and gels.

[0471] Compounds may be incorporated into ointments, which generally are semisolid preparations which are typically based on petrolatum or other petroleum derivatives. The specific ointment base to be used, as will be appreciated by those skilled in the art, is one that will provide for optimum drug delivery, and, preferably, will provide for other desired characteristics as well, e.g., emolliency or the like. As with other carriers or vehicles, an ointment base should be inert, stable, nonirritating and nonsensitizing. As explained in Remington 's, cited in the preceding section, ointment bases may be grouped in four classes:

oleaginous bases; emulsifiable bases; emulsion bases; and water-soluble bases. Oleaginous ointment bases include, for example, vegetable oils, fats obtained from animals, and semisolid hydrocarbons obtained from petroleum. Emulsifiable ointment bases, also known as absorbent ointment bases, contain little or no water and include, for example, hydroxystearin sulfate, anhydrous lanolin and hydrophilic petrolatum. Emulsion ointment bases are either water-in-oil (W/O) emulsions or oil-in-water (O/W) emulsions, and include, for example, cetyl alcohol, glyceryl monostearate, lanolin and stearic acid. Exemplary water-soluble ointment bases are prepared from polyethylene glycols (PEGs) of varying molecular weight; again, reference may be had to Remington's, *supra*, for further information.

[0472] Compounds may be incorporated into lotions, which generally are preparations to be applied to the skin surface without friction, and are typically liquid or semiliquid preparations in which solid particles, including the active agent, are present in a water or alcohol base. Lotions are usually suspensions of solids, and may comprise a liquid oily emulsion of the oil-in-water type. Lotions are preferred formulations for treating large body areas, because of the ease of applying a more fluid composition. It is generally necessary that the insoluble matter in a lotion be finely divided. Lotions will typically contain suspending agents to produce better dispersions as well as compounds useful for localizing and holding the active agent in contact with the skin, e.g., methylcellulose, sodium carboxymethylcellulose, or the like. An exemplary lotion formulation for use in conjunction with the present method contains propylene glycol mixed with a hydrophilic petrolatum such as that which may be obtained under the trademark Aquaphor^{RTM} from Beiersdorf, Inc. (Norwalk, Conn.).

[0473] Compounds may be incorporated into creams, which generally are viscous liquid or semisolid emulsions, either oil-in-water or water-in-oil. Cream bases are water-washable, and contain an oil phase, an emulsifier and an aqueous phase. The oil phase is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol; the aqueous phase usually, although not necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation, as explained in Remington 's, *supra*, is generally a nonionic, anionic, cationic or amphoteric surfactant.

[0474] Compounds may be incorporated into microemulsions, which generally are thermodynamically stable, isotropically clear dispersions of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules (Encyclopedia of

Pharmaceutical Technology (New York: Marcel Dekker, 1992), volume 9). For the preparation of microemulsions, surfactant (emulsifier), co-surfactant (co-emulsifier), an oil phase and a water phase are necessary. Suitable surfactants include any surfactants that are useful in the preparation of emulsions, e.g., emulsifiers that are typically used in the preparation of creams. The co-surfactant (or "co-emulsifer") is generally selected from the group of polyglycerol derivatives, glycerol derivatives and fatty alcohols. Preferred emulsifier/co-emulsifier combinations are generally although not necessarily selected from the group consisting of: glyceryl monostearate and polyoxyethylene stearate; polyethylene glycol and ethylene glycol palmitostearate; and caprilic and capric triglycerides and oleoyl macrogolglycerides. The water phase includes not only water but also, typically, buffers, glucose, propylene glycol, polyethylene glycols, preferably lower molecular weight polyethylene glycols (e.g., PEG 300 and PEG 400), and/or glycerol, and the like, while the oil phase will generally comprise, for example, fatty acid esters, modified vegetable oils, silicone oils, mixtures of mono- di- and triglycerides, mono- and di-esters of PEG (e.g., oleoyl macrogol glycerides), etc.

[0475] Compounds may be incorporated into gel formulations, which generally are semisolid systems consisting of either suspensions made up of small inorganic particles (two-phase systems) or large organic molecules distributed substantially uniformly throughout a carrier liquid (single phase gels). Single phase gels can be made, for example, by combining the active agent, a carrier liquid and a suitable gelling agent such as tragacanth (at 2 to 5%), sodium alginate (at 2-10%), gelatin (at 2-15%), methylcellulose (at 3-5%), sodium carboxymethylcellulose (at 2-5%), carbomer (at 0.3-5%) or polyvinyl alcohol (at 10-20%) together and mixing until a characteristic semisolid product is produced. Other suitable gelling agents include methylhydroxycellulose, polyoxyethylene-polyoxypropylene, hydroxyethylcellulose and gelatin. Although gels commonly employ aqueous carrier liquid, alcohols and oils can be used as the carrier liquid as well.

[0476] Various additives, known to those skilled in the art, may be included in formulations, e.g., topical formulations. Examples of additives include, but are not limited to, solubilizers, skin permeation enhancers, opacifiers, preservatives (e.g., anti-oxidants), gelling agents, buffering agents, surfactants (particularly nonionic and amphoteric surfactants), emulsifiers, emollients, thickening agents, stabilizers, humectants, colorants, fragrance, and the like. Inclusion of solubilizers and/or skin permeation enhancers is particularly preferred, along with emulsifiers, emollients and preservatives. An optimum

topical formulation comprises approximately: 2 wt. % to 60 wt. %, preferably 2 wt. % to 50 wt. %, solubilizer and/or skin permeation enhancer; 2 wt. % to 50 wt. %, preferably 2 wt. % to 20 wt. %, emulsifiers; 2 wt. % to 20 wt. % emollient; and 0.01 to 0.2 wt. % preservative, with the active agent and carrier (e.g., water) making of the remainder of the formulation.

[0477] A skin permeation enhancer serves to facilitate passage of therapeutic levels of active agent to pass through a reasonably sized area of unbroken skin. Suitable enhancers are well known in the art and include, for example: lower alkanols such as methanol ethanol and 2-propanol; alkyl methyl sulfoxides such as dimethylsulfoxide (DMSO), decylmethylsulfoxide (C.sub.10 MSO) and tetradecylmethyl sulfboxide; pyrrolidones such as 2-pyrrolidone, N-methyl-2-pyrrolidone and N-(-hydroxyethyl)pyrrolidone; urea; N,N-diethyl-m-toluamide; C.sub.2 -C.sub.6 alkanediols; miscellaneous solvents such as dimethyl formamide (DMF), N,N-dimethylacetamide (DMA) and tetrahydrofurfuryl alcohol; and the 1-substituted azacycloheptan-2-ones, particularly 1-n-dodecylcyclazacycloheptan-2-one (laurocapram; available under the trademark Azone^{RTM} from Whitby Research Incorporated, Richmond, Va.).

Examples of solubilizers include, but are not limited to, the following: hydrophilic ethers such as diethylene glycol monoethyl ether (ethoxydiglycol, available commercially as Transcutol^{RTM}) and diethylene glycol monoethyl ether oleate (available commercially as Softcutol^{RTM}); polyethylene castor oil derivatives such as polyoxy 35 castor oil, polyoxy 40 hydrogenated castor oil, etc.; polyethylene glycol, particularly lower molecular weight polyethylene glycols such as PEG 300 and PEG 400, and polyethylene glycol derivatives such as PEG-8 caprylic/capric glycerides (available commercially as Labrasol^{RTM}); alkyl methyl sulfoxides such as DMSO; pyrrolidones such as 2-pyrrolidone and N-methyl-2-pyrrolidone; and DMA. Many solubilizers can also act as absorption enhancers. A single solubilizer may be incorporated into the formulation, or a mixture of solubilizers may be incorporated therein.

[0478] Suitable emulsifiers and co-emulsifiers include, without limitation, those emulsifiers and co-emulsifiers described with respect to microemulsion formulations. Emollients include, for example, propylene glycol, glycerol, isopropyl myristate, polypropylene glycol-2 (PPG-2) myristyl ether propionate, and the like.

[0479] Other active agents may also be included in formulations, e.g., other anti-inflammatory agents, analgesics, antimicrobial agents, antifungal agents, antibiotics, vitamins, antioxidants, and sunblock agents commonly found in sunscreen formulations

including, but not limited to, anthranilates, benzophenones (particularly benzophenone-3), camphor derivatives, cinnamates (e.g., octyl methoxycinnamate), dibenzoyl methanes (e.g., butyl methoxydibenzoyl methane), p-aminobenzoic acid (PABA) and derivatives thereof, and salicylates (e.g., octyl salicylate).

[0480] In certain topical formulations, the active agent is present in an amount in the range of approximately 0.25 wt. % to 75 wt. % of the formulation, preferably in the range of approximately 0.25 wt. % to 30 wt. % of the formulation, more preferably in the range of approximately 0.5 wt. % to 15 wt. % of the formulation, and most preferably in the range of approximately 1.0 wt. % to 10 wt. % of the formulation.

[0481] Topical skin treatment compositions can be packaged in a suitable container to suit its viscosity and intended use by the consumer. For example, a lotion or cream can be packaged in a bottle or a roll-ball applicator, or a propellant-driven aerosol device or a container fitted with a pump suitable for finger operation. When the composition is a cream, it can simply be stored in a non-deformable bottle or squeeze container, such as a tube or a lidded jar. The composition may also be included in capsules such as those described in U.S. Pat. No. 5,063,507. Accordingly, also provided are closed containers containing a cosmetically acceptable composition as herein defined.

[0482] In an alternative embodiment, a pharmaceutical formulation is provided for oral or parenteral administration, in which case the formulation may comprises an activating compound-containing microemulsion as described above, but may contain alternative pharmaceutically acceptable carriers, vehicles, additives, etc. particularly suited to oral or parenteral drug administration. Alternatively, an activating compound-containing microemulsion may be administered orally or parenterally substantially as described above, without modification.

[0483] Cells, e.g., treated *ex vivo* with a compound described herein, can be administered according to methods for administering a graft to a subject, which may be accompanied, e.g., by administration of an immunosuppressant drug, e.g., cyclosporin A. For general principles in medicinal formulation, the reader is referred to Cell Therapy: Stem Cell Transplantation, Gene Therapy, and Cellular Immunotherapy, by G. Morstyn & W. Sheridan eds, Cambridge University Press, 1996; and Hematopoietic Stem Cell Therapy, E. D. Ball, J. Lister & P. Law, Churchill Livingstone, 2000.

[0484] Also provided herein are kits, e.g., kits for therapeutic and/or diagnostic purposes. A kit may include one or more compounds described herein, and optionally devices for

contacting tissue or cells with the compounds. Devices include needles, syringes, stents, resuspension liquid, and other devices for introducing a compound into a subject.

[0485] In any of the forgoing embodiments 1,5-*bis*(*p*-aminophenoxy)pentane may be specifically excluded.

[0486] In any of the forgoing embodiments 11-*cis*-retinol may be specifically excluded.

[0487] In any of the forgoing embodiments 11-*cis*-retional palmitate may be specifically excluded.

[0488] In any of the forgoing embodiments 13-*cis*-retinoic acid (accutane) may be specifically excluded.

[0489] In any of the forgoing embodiments 2-bromopalmitic acid may be specifically excluded.

[0490] In any of the forgoing embodiments 3-aminobenzoic acid ethyl ester methane sulfonate may be specifically excluded.

[0491] In any of the forgoing embodiments acetaminophen may be specifically excluded.

[0492] In any of the forgoing embodiments adamantylamine may be specifically excluded.

[0493] In any of the forgoing embodiments all-*trans*-retinaldehyde may be specifically excluded.

[0494] In any of the forgoing embodiments all-*trans*-retinoic acid may be specifically excluded.

[0495] In any of the forgoing embodiments all-*trans*-retinol (vitamin A) may be specifically excluded.

[0496] In any of the forgoing embodiments all-*trans*-retinyl plamitate may be specifically excluded.

[0497] In any of the forgoing embodiments analine may be specifically excluded.

[0498] In any of the forgoing embodiments cyclohexylamine may be specifically excluded.

[0499] In any of the forgoing embodiments dapson may be specifically excluded.

[0500] In any of the forgoing embodiments diaminophenoxypentane may be specifically excluded.

[0501] In any of the forgoing embodiments ethyl *m*-aminobenzoate may be specifically excluded.

[0502] In any of the forgoing embodiments *m*-aminobenzoic acid may be specifically excluded.

[0503] In any of the forgoing embodiments *m*-phenetidine may be specifically excluded.

[0504] In any of the forgoing embodiments *N*-(4-hydroxyphenyl)retinamide (fenretinide) may be specifically excluded.

[0505] In any of the forgoing embodiments *N*,*N*-dimethylaniline may be specifically excluded.

[0506] In any of the forgoing embodiments *N*,*N*-dimethyl-*p*-phenetidine may be specifically excluded.

[0507] In any of the forgoing embodiments *N*-methylaniline may be specifically excluded.

[0508] In any of the forgoing embodiments *N*-methyl-*p*-phenetidine may be specifically excluded.

[0509] In any of the forgoing embodiments *o*-phenetidine may be specifically excluded.

[0510] In any of the forgoing embodiments *p*-(n-hexyloxy)aniline may be specifically excluded.

[0511] In any of the forgoing embodiments *p*-(n-hexyloxy)benzamide may be specifically excluded.

[0512] In any of the forgoing embodiments *p*-(n-hexyloxy)benzoic acid hydrazide may be specifically excluded.

[0513] In any of the forgoing embodiments *p*-anisidine may be specifically excluded.

[0514] In any of the forgoing embodiments *p*-ethylanaline may be specifically excluded.

[0515] In any of the forgoing embodiments *p*-ethyoxybenzylamine may be specifically excluded.

[0516] In any of the forgoing embodiments *p*-ethyoxyphenol may be specifically excluded.

[0517] In any of the forgoing embodiments phenetidine may be specifically excluded.

[0518] In any of the forgoing embodiments piperidine may be specifically excluded.

[0519] In any of the forgoing embodiments *p*-n-boutoxyaniline may be specifically excluded.

[0520] In any of the forgoing embodiments *p*-n-butylaniline may be specifically excluded.

[0521] In any of the forgoing embodiments *p*-n-dodecylaniline may be specifically excluded.

[0522] In any of the forgoing embodiments *p*-nitroaniline may be specifically excluded.

[0523] In any of the forgoing embodiments sulfabenzamide may be specifically excluded.

[0524] In any of the forgoing embodiments sulfamoxaole may be specifically excluded.

[0525] In any of the forgoing embodiments sulfanilamide may be specifically excluded.

[0526] In any of the forgoing embodiments tricaine may be specifically excluded.

[0527] In addition, any compound cited in the references incorporated herein may also be specifically excluded from any of the forgoing embodiments.

[0528] **4. Methods**

[0529] Disclosed herein are methods for treating or preventing an ophtalmologic disorder. An exemplary method comprises administering to a subject in need thereof a therapeutically effective amount of a composition, e.g., a pharmaceutical composition, described herein. A subject in need thereof may be a subject who knows that he has or is likely to develop an opthalmologic disorder.

[0530] As discussed above, a disclosed composition may be administered to a subject in order to treat or prevent macular degeneration. Other diseases, disorders, or conditions characterized by the accumulation of retinotoxic compounds in the RPE may be similarly treated.

[0531] In one embodiment, a drug is administered to a subject that short-circuits the visual cycle at a step of the visual cycle that occurs outside a disc of a rod photoreceptor cell. For example, as shown in Figure 3, the drug may react with 11-*cis*-retinal in the RPE and shunt it to all-*trans*-retinal while it remains in the RPE. More specifically, the therapeutic may react with 11-*cis*-retinal to form an intermediate that isomerizes to the all-*trans* configuration. The all-*trans* intermediate may then release the therapeutic to form all-*trans*-retinal. The all-*trans*-retinal could then be re-processed through the remainder of the visual cycle as normal in the RPE. Thus, the visual cycle would be reduced to a futile cycle, in which all-*trans*-retinal has little or no opportunity to accumulate in the disc.

[0532] In one embodiment, a subject may be diagnosed as having macular degeneration, and then a disclosed drug may be administered. In another embodiment, a subject may be identified as being at risk for developing macular degeneration (risk factors include a history of smoking, age, female gender, and family history). In yet another embodiment, a subject may be diagnosed as having Stargardt's disease, a familial form of macular degeneration. In some embodiments, a drug may be administered prophylactically. In some embodiments, a subject may be diagnosed as having the disease before retinal damage is apparent. For example, a subject may be found to carry a gene mutation for *abcr, elovl4*, and/or another gene, and thus be diagnosed as having Stargardt's disease before any ophthalmologic signs are manifest, or a subject may be found to have early macular changes indicative of macular degeneration before the subject is aware of any effect on

vision. In some embodiments, a human subject may know that he or she is in need of the macular generation treatment or prevention.

[0533] In some embodiments, a subject may be monitored for the extent of macular degeneration. A subject may be monitored in a variety of ways, such as by eye examination, dilated eye examination, fundoscopic examination, visual acuity test, angiography, fluorescein angiography, and/or biopsy. Monitoring can be performed at a variety of times. For example, a subject may be monitored after a drug is administered. The monitoring can occur one day, one week, two weeks, one month, two months, six months, one year, two years, and/or five years after the first administration of a drug. A subject can be repeatedly monitored. In some embodiments, the dose of a drug may be altered in response to monitoring.

[0534] In some embodiments, the disclosed methods may be combined with other methods for treating or preventing macular degeneration, such as photodynamic therapy.

[0535] In some embodiments, a drug for treating or preventing macular degeneration may be administered chronically. The drug may be administered daily, more than once daily, twice a week, three times a week, weekly, biweekly, monthly, bimonthly, semiannually, annually, and/or biannually.

[0536] The therapeutics may be administered by a wide variety routes, described above. In some embodiments, a drug may be administered orally, in the form of a tablet, a capsule, a liquid, a paste, and/or a powder. In some embodiments, a drug may be administered locally, as by intraocular injection. In some embodiments, a drug may be administered systemically in a caged, masked, or otherwise inactive form and activated in the eye (such as by photodynamic therapy). In some embodiments, a drug may be administered in a depo form, so sustained release of the drug is provided over a period of time, such as hours, days, weeks, and/or months.

[0537] The therapeutic agents are used in amounts that are therapeutically effective, which varies widely depending largely on the particular agent being used. The amount of agent incorporated into the composition also depends upon the desired release profile, the concentration of the agent required for a biological effect, and the length of time that the biologically active substance has to be released for treatment. In certain embodiments, the biologically active substance may be blended with a compound matrix at different loading levels, in one embodiment at room temperature and without the need for an organic solvent.

In other embodiments, the compositions may be formulated as microspheres. In some embodiments, the drug may be formulated for sustained release.

[0538] It is noted that disruption of the visual cycle to prevent accumulation of A_2E may impair a subject's night (low-light) vision and might cause night-blindness. Indeed, some of the therapeutics noted herein as appropriate for preventing A_2E accumulation have been used sparingly in humans or withheld from use entirely because of their propensity to cause night-blindness. However, with the recognition that this very cause of night blindness might be turned to the therapeutic and/or preventative treatment of macular degeneration, it is likely that patients in need of such treatment would readily accept some night-blindness in return for sparing of normal vision. This is because the visual cycle described above operates in rod photoreceptors, which operate only at low levels of illumination and do not operate during the day. Therefore, macular function would be little affected by decreases in visual cycle function, while there might be some effect on low light vision at night. At least some patients, and probably most, might readily sacrifice a decrement in night vision for a lessening of the probability that they would eventually lose their cone day vision.

[0539] *Palmitoylation*

[0540] In some embodiments, inhibitors of LRAT can be used to modulate palmitoylation of RPE65. RPE65 occurs in at least two forms, membrane-associated (mRPE65) and soluble (sRPE65). As discussed in greater detail below, mRPE65 is a palmitoylated form of RPE65, and sRPE65 is a depalmitoylated form.

[0541] The flux of retinoids in the visual cycle can be regulated by the reversible palmitoylation of RPE65 by LRAT. mRPE65 specifically binds long chain all-*trans*-retinyl esters and mobilizes them for further processing in the visual cycle. The all-*trans*-retinyl esters are the substrates for the IMH, which converts them into 11-*cis*-retinol. An all-*trans*-retinyl ester chaperone role for mRPE65 is required for mobilization of these esters. Regulation is not implicit here because there is no molecular alteration of mRPE65 implied during the operation of the cycle. Several observations reported here, however, bear on this issue and make it exceedingly likely that regulation is imposed on the visual cycle at the RPE65 stage.

[0542] The salient facts with respect to invoking regulation at the level of RPE65 can be summarized as follows: (1) mRPE65 and sRPE65 show different and complementary retinoid binding profiles. mRPE65 specifically binds all-*trans*-retinyl esters and makes them available for IMH processing, while sRPE65 specifically binds vitamin A, making it

available for LRAT. (2) the predominant form of RPE65 as isolated is sRPE65, and not mRPE65. (3) mRPE65 and sRPE65 differ in their states of palmitoylation. (4) the reversible sRPE65 to mRPE65 interconversion is cooperative and catalyzed by LRAT, so that small changes in the levels of mRPE65 will have a magnified effect on isomerization. (5) mRPE65 acts as a palmitoyl donor for 11-*cis*-retinol in the presence of LRAT, revealing a dual role for mRPE65, as a retinoid binding protein and an acyl donor which limits isomerization by decreasing the levels of mRPE65, and (6) all-*trans*-retinyl esters have the opposite effect, because they drive sRPE65 to mRPE65.

[0543] A simple working model can be generated to synthesize the experimental observations made here into an important regulatory element in the control of the visual cycle. Figures 13A-B show how the regulatory elements described might direct the flow of retinoids in vision. In the dark, when formation of the visual chromophore 11-*cis*-retinal is not required, sRPE65 is expected to be the predominant form of RPE65. The sRPE65 is generated by the palmitoylation of 11-*cis*-retinol by mRPE65, and perhaps also by the hydrolysis of mRPE65 by palmitoyl esterases activated in the dark. It is quite conceivable that G-protein coupled events are involved here. Light flips the switch (Figure 13A), because the photoisomerization of rhodopsin in the photoreceptors results in a flux of vitamin A to the RPE. The RPE is primed to chaperone vitamin A to LRAT to generate all-*trans*-retinyl esters, the substrates for IMH. The all-*trans*-retinyl esters have a second role, as shown here, to drive the sRPE65 to mRPE65 conversion. This process is cooperative, so that small changes in the concentration of mRPE65 will have large effects on the rate of processing of all-*trans*-retinyl esters and isomerization. The mRPE65 directs the flow of all-*trans*-retinyl esters to IMH, where it is processed to form 11-*cis*-retinol. Once the 11-*cis*-retinol is formed, it can be partitioned directly into 11-*cis*-retinal, the chromophore of rhodopsin, by binding to cRALBP, with subsequent oxidation by 11-*cis*-retinol dehydrogenase. This flow of chromophore occurs to the photoreceptors when opsin is made available as a consequence of the bleaching of rhodopsin in the light. The exothermic binding of opsin with 11-*cis*-retinal to form rhodopsin drives this process.

[0544] The switch would be turned back off in the dark because 11-*cis*-retinol is palmitoylated, using mRPE65 as the acyl donor to form 11-*cis*-retinyl palmitate, the storage form of the chromophore, and sRPE65. This shuts the system down, because the latter is a chaperone for vitamin A, not all-*trans*-retinyl esters, and is unable to facilitate IMH processing. Again, because of the cooperativity of the process, a small shift in

concentration of mRPE65 will have a large effect on the rate of 11-*cis*-retinol synthesis. The palmitoylation of 11-*cis*-retinol by mRPE65 also would explain the putative turnover of mRPE65 during the operation of the visual cycle, although as suggested above, additional factors may also enhance mRPE65 hydrolysis. Thus, the proposed switch would operate very simply: the rise in all-*trans*-retinyl ester levels facilitates chromophore biosynthesis because mRPE65 is regenerated to direct retinoid flow to the IMH. The rise in 11-*cis*-retinol formation switches off the system because it drives the mRPE65 to sRPE65 conversion. It is already known that added 11-*cis*-retinol is a powerful inhibitor of chromophore biosynthesis *in vivo*, and it is shown here in Figure 12A that this inhibition is at least in part due to the switch effect. Finally, the existence of this switch-based regulatory element is also consistent with the observation that 11-*cis*-retinoid regeneration in the dark is a very sluggish affair.

[0545] The studies described here are of general interest beyond their impact on visual processing. Certainly, palmitoyl switch mechanisms could operate in a variety of signal transduction contexts, in addition to the one explored here. On a biochemical level the molecular basis of the differences in ligand binding selectivity between mRPE65 and sRPE65 are related only to differences in their extents of palmitoylation. Protein palmitoylation represents a well-known post-translational modification, whose principle roles are to enhance the hydrophobicity of proteins, targeting them to membranes, and also to enhance protein-protein interactions in certain cases. There is no doubt that in the case of RPE65 palmitoylation-mediated transition of sRPE65 to mRPE65, membrane targeting is an outcome. However, the studies reported here reveal two other roles for palmitoylation. First, as mentioned above, palmitoylation alters the ligand binding specificity of the modified protein. Whether the palmitoyl group(s) of mRPE65 directly interacts with the all-*trans*-retinyl esters, thus enhancing binding for these molecules through hydrophobic interactions, or whether palmitoylation causes a conformational change in the protein is currently unknown. Second, we also show that a palmitoylated protein (mRPE65) can function as a palmitoyl donor. Reversible palmitoylation has been described and this reversibility may be of regulatory significance (Houslay 1996; Mumby 1997; Bijlmakers and Marsh 2003; Qanbar and Bouvier 2003). This is especially interesting in signal transduction processes where small G proteins are palmitoylated (Milligan 1995; Morello 1996; Mumby 1997; Resh 1999; Chen and Manning 2001; El-Husseini and Bredt 2002; Bijlmakers and Marsh 2003; Qanbar and Bouvier 2003). In

these cases, removal of a palmitoyl moiety is thought to occur by means of an esterase, but an acyl carrier role for the small G-proteins may not have been addressed (Mumby 1997; Resh 1999; Linder and Deschenes 2003).

[0546] LRAT catalyzes the interconversion of mRPE65 and sRPE65, and hence this enzyme is bi-functional because it is also responsible for the bulk synthesis of all-*trans*-retinyl esters in the visual cycle. In the studies reported here, mRPE65 acts as the palmitoyl donor, rather than lecithin. This result is surprising because hitherto LRAT had been considered a rather narrowly specific enzyme that used lecithin (i.e. DPPC) as an acyl donor and a retinol as the acyl acceptor (Cañada et al., 1990; Barry et al., 1989; Saari 2000). With respect to acyl donor function, neither the phosphatidylethanolamines nor the phosphatidylserines substitute for lecithin (Cañada et al., 1990).

[0547] LRAT is the founder member of an expanding group of proteins, many of which are of unknown function (Jahng et al., 2003b). The proteins of unknown function include class II tumor suppressors and EGL-26, a putative enzyme that mediates morphogenesis in *C. elegans* (Hanna-Rose 2002; Anantharaman and Arvind 2003). These proteins should be considered as possible palmitoyl transferase candidates. Along these lines, it is interesting to note that the identification of dedicated palmitoyl transferase enzymes has not been forthcoming, and the possibility of chemical, rather than enzymatic palmitoylation, is a considered alternative (Mumby 1997; Resh 1999; Linder and Deschenes 2003; Bijlmakers and Marsh 2003). Accordingly, compounds identified herein as modulators or inhibitors of LRAT may be considered prototypical palmitoyl transferase modulators or inhibitors, and may be used to modulate other palmitoyl transferases in the expanding LRAT class.

[0548] 5. Screening methods

[0549] Suitable drugs may be identified by a variety of screening methods. For example, a candidate drug may be administered to a subject that has or is at risk for having macular degeneration, e.g., an animal that is an animal model for macular degeneration, and the accumulation of a retinotoxic compound, such as A_2E, can be measured. A drug that results in reduced accumulation of a retinotoxic compound compared to a control (absence of the drug) would thus be identified as a suitable drug. Alternatively, photoreceptor disks may be analyzed for the presence of all-*trans*-retinal, *N*-retinylidene-PE, and/or A_2E. Animal models that have rapid development of macular degeneration are of considerable interest because naturally-occurring macular degeneration typically takes years to develop. A number of animal models are accepted models for macular degeneration. For example, the

abcr -/- knockout mouse has been described as a model for macular degeneration and/or lipofuscin accumulation, as has been the *elovl4* -/- knockout mouse. In addition, knockout mice deficient in monocyte chemoattractant protein-1 (Ccl-2; also known as MCP-1) or it cognate receptor, C-C chemokine receptor-2 (Ccr-2), have also been described as accelerated models for macular degeneration.

[0550] In addition, *in vitro* models of the visual system may facilitate screening studies for drugs that inhibit or short circuit the visual cycle. *In vitro* models can be created by placing selected intermediates in solution with appropriate enzymes and other necessary cofactors. Alternatively, an *in vitro* RPE culture system may be employed. For example, LRAT inhibition can be tested by adding a candidate drug to a solution containing LRAT and a substrate for LRAT, and measuring accumulation of an expected product. Analogous systems are envisioned for the other potential inhibition targets described herein.

[0551] The practice of the present methods will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See, for example, Molecular Cloning A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989); DNA Cloning, Volumes I and II (D. N. Glover ed., 1985); Oligonucleotide Synthesis (M. J. Gait ed., 1984); Mullis et al. U.S. Patent No: 4,683,195; Nucleic Acid Hybridization (B. D. Hames & S. J. Higgins eds. 1984); Transcription And Translation (B. D. Hames & S. J. Higgins eds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu et al. eds.), Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and C. C. Blackwell, eds., 1986); Manipulating the Mouse Embryo, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

EXAMPLES

[0552] The present description is further illustrated by the following examples, which should not be construed as limiting in any way. The contents of all cited references

(including literature references, issued patents, published patent applications as cited throughout this application) are hereby expressly incorporated by reference.

[0553] Example 1: in vitro

[0554] Materials: Frozen bovine eyecups devoid of retinas were purchased from W. L. Lawson Co., Lincoln, NE. Ammonium bicarbonate, BSA, ethylenediaminetetraacetic acid (EDTA), guanidine HCl, imidazole, DEAE-Sepharose, phenyl-Sepharose CL–4B, all-*trans*-retinol, all-*trans*-retinyl palmitate, α-Cyano-4-hydroxycinnamic acid and Trizma® base were from Sigma-Aldrich. Dithiothreitol was from ICN Biomedicals Inc. 11-*cis*-Retinol and 11-*cis*-retinyl palmitate were synthesized by following the procedure described elsewhere (Shi et al., 1993). Anagrade™ CHAPS and dodecyl maltoside were from Anatrace. HPLC grade solvents were from Sigma-Aldrich Chemicals. Anti RPE65 (NFITKVNPETLETIK) antibody was obtained from Genmed Inc and anti-LRAT antibody was provided by Prof. Dean Bok (University of California at Los Angeles). rHRPE65 baculovirus was provided by Prof. Jian-Xin Ma (University of South Carolina). Hank's TNM-FH Insect medium was obtained from JRH Biosciences. *sf*21cells were laboratory stock from Prof. Steven Harrison's laboratory (Harvard Medical School). Broad spectrum EDTA-free protease inhibitor cocktail was obtained from Roche Biosciences. Nickel-NTA resin and Nickel-NTA spin column were purchased from Qiagen Inc. The precast gels (4-20%) for sodium dodecylsulfate-polyacrlyamide gel electrophoresis, BenchMark prestained and Magic molecular weight markers were from Invitrogen. DEAE Sepharose was from Amersham Biosciences. Buffers were changed by dialysis in the request buffer overnight in a slide-a-lyser™ cassette from Pierce (10 KDa MWCO). RPE65 solutions were concentrated with an Amicon Ultra™ centrifugal filtration device (30 KDa-cutoff) from Millipore Corp. All reagents were analytical grade unless specified otherwise.

[0555] Methods

[0556] Purification of mRPE65, sRPE65 and rHRPE65: Purification was performed as described before (Ma et al., 2001). The purities of these proteins were verified by silver staining or Coomassie staining and Western blot (1:4000 primary antibody-1h at room temperature and 1:4000 secondary antibody-0.5h at room temperature).

[0557] Purification of rCRALBP: Purification was performed as previously described (25). The purities of these proteins were verified by silver staining or Coomassie staining and Western blot analysis (1:4000 primary antibody-1h at room temperature and 1:4000 secondary antibody-0.5h at room temperature).

[0558] Fluorescence binding assays: RPE65 in PBS, 1% CHAPS, pH 7.4 was used in the fluorometric titration studies. Protein concentrations were measured by a modified Lowry method (Lowry et al., 1951). All titrations were performed at 25°C. The samples in PBS buffer were excited at 280 nm and the fluorescence was scanned from 300 to 500 nm. Fluorescence measurements, using 450 µL quartz cuvettes with a 0.5 cm path length, were made at 25°C on a Jobin Yvon Instruments, Fluoromax 2 employing the right–angle detection method.

[0559] The fluorescence of the protein solution was measured after equilibrating it at 25°C for 10 min. The sample was then titrated with a solution of retinoid dissolved in dimethyl sulfoxide. In each titration, to a 250 µL solution of the protein an equal amount of retinoid, typically 0.2 µL. was added and thoroughly mixed before allowing it to equilibrate for 10 min prior to recording the fluorescence intensity. The addition of dimethyl sulfoxide (0.1% per addition) did not have any effect on the fluorescence intensity. The binding constant (K_D) was calculated from the fluorescence intensity by using the following equation (Gollapalli et al., 2003).

$$P_0\alpha = \frac{R_0\alpha}{n(1-\alpha)} - \frac{K_D}{n}$$

where P_0 = Total protein concentration, $\alpha = \frac{F_{max} - F}{F_{max} - F_0}$, n = number of independent binding sites, R_0 = Total retinoid concentration at each addition, K_D = dissociation constant, F_{max} = Fluorescence intensity at saturation, and F_0 = Initial fluorescence intensity.

[0560] Competitive binding of retinoic acid (all-*trans* and 13-*cis*) and all-*trans*-retinyl palmitate to RPE65: Buffer exchange experiments were performed to investigate the abilities of the retinoic acids (all-*trans* and 13-*cis*) to displace all-*trans*-retinyl palmitate binding from RPE65. To RPE65 (0.5 µM) (PBS, 1% CHAPS, pH 7.4), was added 6 µM of retinoic acid (all-*trans* and 13-*cis*) and incubated at 4°C for 30 min. A control sample of RPE65 was incubated minus retinoic acids at 4°C for 30 min. At the end of this incubation, the samples were incubated for 30 min with ^{3}H-all-*trans*-retinyl palmitate (0.65 µM, 20.31 Ci/mmol). At the end of this incubation period the buffer (PBS-1% CHAPS) was exchanged 10^4 fold with a Centricon 30K MWCO filter. The sample retained and the buffer flow through were counted on a liquid scintillation counter, to measure the amount of ^{3}H-all-*trans*-retinyl palmitate retained.

[0561] Effect of all-*trans* Retinoic acid (atRA), 13-*cis*-Retinoic acid (13cRA) and N-(4-hydroxyphenyl)retinamide (4-HPR) on IMH: To 1mL of buffered suspension of RPE membranes (100 mM Tris pH 8.0, 76.7 μg of protein) was added 60 μM or 6 μM of atRA, 13cRA or 4-HPR and incubated at room temp. for 15 min. A control reaction mixture without any inhibitor was also incubated at room temperature for 15 min. At the end of the 15 min incubation, all-*trans*-retinol [11-12-$^3H_2$] (0.2 μM) was added to the reaction mixtures (100mM Tris pH 8.0, 76.7 μg of RPE protein, 0.2 % BSA 100 μM of DPPC, 1 mM of DTT and 0.2 μM all-*trans*-retinol [11-12-$^3H_2$]) and incubated at room temperature for 30 min. At the end of this 30 minutes of incubation, an aliquot of the reactions were quenched to verify the equal addition of all-*trans*-retinol [11,12-$^3H_2$] and the effect of these inhibitors on LRAT. After this the control reaction mixture was incubated with atRA (60 & 6 μM), 13cRA (60 & 6 μM) or 4-HPR (60 & 6 μM) for 15 min. Now all the reaction mixtures were incubated with 30 μM of apo-rCRALBP (100 mM Tris pH 8.0, 7.7 μg of RPE protein, 0.2 % BSA 100 μM of DPPC, 1 mM of DTT 30 μM apo-rCRALBP and 0.2 μM all-*trans*-retinol [11-12-$^3H_2$]) at 37^{0}C for 30 minutes. At the end of this incubation period the 200 μL reaction mixture was quenched by the addition of 750 μL ice cold methanol after which 100 μL of 1M sodium chloride solution was added, and 500 μl hexane (containing butylated hydroxy toluene at 1 mg/mL) was added to effect extraction of the retinoids. The retinoids were analyzed as previously described (27). The amount of 11-*cis*-retinol formed was used as a measurement of IMH activity. All experiments were performed in triplicate and the average values of these measurements were used for analysis.

[0562] $^3H_2$ Palmitoylation of rHmRPE65: 6xHis-recombinant human membrane associated RPE65 was expressed in recombinant baculovirus in *sf*21 insect cells. The *sf*21 cells were transfected with recombinant baculovirus followed by incubation for 8hrs at 25°C, followed by addition of (0.09 μM) of $^3H_2$ palmitic acid (0.5 mCi/mL). The culture was incubated at 25°C for 48h. A similar culture with non-radioactive palmitic acid (0.09 μM) was grown as control. At the end of the expression, the cells were harvested at 500xg. The cells were lysed in 100 mM phosphate buffer with 500 mM NaCl-pH8.0, 5mM imidazole and 6 M guanidine HCl. The lysis buffer contained the appropriate amount of protease inhibitor cocktail as per the manufacture's instructions. The lysed cells were then centrifuged at 100,000xg to pellet the cell debris, and purified on a Nickel-NTA column following the manufactures instructions. The purified protein solution was divided into two parts: (1) was

treated for 16 h with 0.5 M Tris pH 8.0 and (2) was treated for 16 h with 0.5 M hydroxyl amine pH 8.0. The protein samples were then analyzed by sodium dodecylsulfate-polyacrlyamide gel electrophoresis, Western blot analysis, and autoradiography.

[0563] MALDI-TOF analysis of purified bovine mRPE65 and sRPE65: MALDI-TOF mass analysis was performed using a Voyager-DE STR from Applied Biosystems. mRPE65 and sRPE65 were purified as described above. The gel band containing pure mRPE65 and sRPE65 was dehydrated in acetonitrile for 10 min. Gel pieces were covered with dithiothreitol (10 mM) in ammonium bicarbonate (100 mM) to reduce the proteins for 1 h at 56 °C. After cooling to room temperature, the reducing buffer was removed. The gel washing/dehydration cycle was repeated 3 times with ammonium bicarbonate/acetonitrile before trypsin (12.5 ng/μL, 5 μL/mm^2 gel, overnight) digestion at 37 °C. Gel slices were centrifuged and the supernatant was collected. Peptides were further extracted by one change of 20 mM ammonium bicarbonate and three changes 50% acetonitrile (20 minutes between changes) at 25 °C. α-Cyano-4-hydroxycinnamic acid (0.5 μL, 10 mg/mL) was used as the matrix for each sample (0.5 μL). Samples were run in the reflector mode with 20000V of accelerating voltage and 200 nsec of extraction delay time. The laser intensity was 1900-2300 and 100-200 laser shots were collected for each spectrum. The acquisition mass range was 750-4500 Da with a 600 Da low mass gate.

[0564] Effect of sRPE65 on tLRAT mediated esterification: The activity of LRAT was determined by monitoring the formation of tLRAT catalyzed retinyl esters from added all-*trans*-retinol [11,12-$^3H_2$] sRPE65 and/or DPPC/ dodecyl maltoside. In all of the studies reported here truncated LRAT (tLRAT) is used (Jahng et al., 2003b). This form of LRAT has the two N and C-terminal transmembrane domains of LRAT truncated, and is His-tagged which allows for the bacterial expression of LRAT and for its full purification (Bok et al., 2003). LRAT has never been purified and is not expressible in bacteria. Kinetic studies on LRAT and tLRAT show them to behave identically (Bok et al., 2003). In the current experiments, the reaction mixture (volume 0.1 mL) contains 100 mM Tris (pH 8.4), 5 μM of tLRAT, 200 μM DPPC/0.1% dodecyl maltoside and/or 0.04 μM sRPE65, 1 mM dithiothreitol and 0.2 μM of all-*trans*-retinol [11,12-$^3H_2$] and incubated for 10 min at room temperature. After 10 min the reaction was quenched with 500 μL methanol, 100 μL of water and 500 μL of hexane. The amount of all-*trans*-retinyl palmitate [11,12-$^3H_2$] formed as determined by normal phase HPLC and was used as a measure of activity. Each

experiment was done in duplicate, and the data points used are an average of these two points.

[0565] mRPE65 concentration-dependent esterification of vitamin A: The effect of mRPE65 concentration on the rates of all-*trans*-retinyl palmitate formation was determined by monitoring the tLRAT catalyzed formation of all-*trans*-retinyl palmitate from added [11,12-$^3H_2$]-all-*trans*-retinol and mRPE65. It should be noted that all-*trans*-retinyl palmitate formed from mRPE65 and vitamin A was identified both by its mass spectroscopic and chromatographic properties. The reaction mixture (volume 0.1 mL) contains 100 mM Tris (pH 8.4), 5 μM of tLRAT 0.3% CHAPS, 1 mM dithiothreitol and 5 μM of all-*trans*-retinol [11,12-$^3H_2$] and mRPE65 (0, 0.008, 0.02, 0.028, 0.04, 0.052, 0.06 and 0.08 μM) incubated for 10 min at room temperature. After 10 min the reaction was quenched with 500 μL methanol, 100 μL of water and 500 μL of hexane. The amount of all-*trans*-retinyl palmitate [11,12-$^3H_2$] formed as determined by normal phase-HPLC and was used as a measure of activity. The kinetic paramaters K_M (K_{app}) and N were calculated as described before (Segal 1993). Each experiment was done in triplicate, and the data points used are an average of these three points. The standard errors are presented as error bars.

[0566] RPE65 mediated reversible palmitoylation of vitamin A: The reversibility of the palmitoylation of vitamin A, mediated by tLRAT in the presence of RPE65 was investigated. A reaction mixture consisting of 100 mM Tris pH 8.4, 0.06 μM of mRPE65, 1 mM dithiothreitol, 1 mM EDTA 5 μM of tLRAT and 5 μM of all-*trans*-retinol was incubated for 1hr. This was followed by addition of 5 μM of all-*trans*-retinol [11,12-$^3H_2$] (4.05 Ci/mmol). Aliquots were removed from the reaction after 0, 2, 7, 10, 20 and 35 min and the reaction was quenched by the addition of 500 μL of methanol, 100 μL of H_2O and extracted with 500 μL of hexane. The all-*trans*-retinyl esters were separated from all-*trans*-retinol and the specific activities for each fraction was calculated as described before (Gollapalli and Rando 2003). Each time point was done in triplicates and the average value was used. The standard errors are presented as error bars.

[0567] *In vitro* conversion of mRPE65 to sRPE65: Purified mRPE65 (0.02 μM) was incubated with tLRAT (5 μM) and all-*trans*-retinol (0.2 μM) at room temperature for 2h. At the end of the reaction the reaction mixture was irradiated with UV light (365 nm) for 15 min to destroy the endogenous retinoids. The solution was dialyzed against a dialysis buffer containing 100 mM phosphate buffer (pH 8.0), 500 mM NaCl, 5 mM imidazole and 1% CHAPS. The dialyzed reaction mixture was then concentrated and passed through a

Nickel-NTA spin column to remove the 6xHis tagged tLRAT. The flow through was concentrated and used in the fluorescence binding assay as described above. The removal of tLRAT was confirmed by Western blot (1:4000 primary antibody-1hr analysis at room temperature and 1:4000 secondary antibody–0.5hr at room temperature).

[0568] Isomeric preference of the mRPE65/tLRAT mediated esterification of retinols: The effect of mRPE65 on the processing of 11-*cis*-and all-*trans*-retinols was determined by monitoring the formation of tLRAT catalyzed retinyl esters from added all-*trans*-retinol [11,12-$^3H_2$], 11-*cis*-retinol [15-^3H] and mRPE65. The reaction mixture (volume 0.1 mL) contains 100 mM Tris (pH 8.4), 5 μM of tLRAT 0.3% CHAPS, 1 mM dithiothreitol and 0.2 μM of all-*trans*-retinol [11,12-$^3H_2$] or 11-*cis*-retinol [15-^3H] and mRPE65 (0.02 μM) or 200 μM/0.4 % DPPC/BSA was incubated for 10 min at room temperature. After 10 min the reaction was quenched with 500 μL methanol, 100 μL of water and 500 μL of hexane. The amount of retinyl palmitate formed, as determined by normal phase-HPLC, was used as a measure of activity. Each experiment was done in triplicate, and the data points used are an average of these three points. The standard error was presented as error bars.

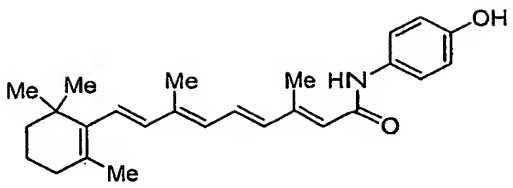
[0569] Effect of 11-*cis*-retinol mediated depalmitoylation of mRPE65 on the generation of 11-*cis*-retinol: To 1mL of buffered suspension of RPE membranes (100 mM Tris pH 8.0, 80 μg of protein) was added 10 μM of 11-*cis*-retinol and incubated at room temp. for 45 min. A control reaction mixture without 11-*cis*-retinol was also incubated at room temperature for 45 min. At the end of the 45 min incubation, the reaction mixtures were exposed to UV light (354 nm) for 10 min to destroy the 11-*cis*-retinoids. All-*trans*-retinol [11-12-$^3H_2$] (0.1 μM) was then added to the reaction mixtures (100mM Tris pH 8.0, 80 μg of RPE protein 5 % BSA and 0.1 μM all-*trans*-retinol [11-12-$^3H_2$], and incubated at 37°C. 100 μL aliquot of the reactions were quenched after 0, 5, 10, 15, 20, 30, 45, 60, 90, 120 and 150 min by the addition of 500 μL methanol after which 100 μL of H_2O was added, and 500 μl hexane (containing butylated hydroxy toluene at 1 mg/mL) was added to effect extraction of the retinoids. The retinoids were analyzed as previously described (Winston and Rando 1998). The amount of 11-*cis*-retinol formed was used as a measurement of IMH activity. All experiments were performed in triplicate and the average values of these measurements were used for analysis.

[0570] A. Blocking RPE65 binding to retinyl esters

[0571] Effect of all-*trans* Retinoic acid (atRA), 13-*cis*-Retinoic acid (13cRA) and N-(4-hydroxyphenyl)retinamide (4-HPR) on IMH: To 1mL of buffered suspension of RPE

membranes (100 mM Tris pH 8.0, 76.7 μg of protein) was added 60 μM or 6 μM of atRA, 13cRA or 4-HPR and incubated at room temp. for 15 min. A control reaction mixture without any inhibitor was also incubated at room temperature for 15 min. At the end of the 15 min incubation, all-*trans*-retinol [11-12-$^3H_2$] (0.2 μM) was added to the reaction mixtures (100mM Tris pH 8.0, 76.7 μg of RPE protein, 0.2 % BSA 100 μM of DPPC, 1 mM of DTT and 0.2 μM all-*trans*-retinol [11-12-$^3H_2$]) and incubated at room temperature for 30 min. At the end of this 30 minutes of incubation, an aliquot of the reactions were quenched to verify the equal addition of all-*trans*-retinol [11,12-$^3H_2$] and the effect of these inhibitors on LRAT. After this the control reaction mixture was incubated with atRA (60 & 6 μM), 13cRA (60 & 6 μM) or 4-HPR (60 & 6 μM) for 15 min. Now all the reaction mixtures were incubated with 30 μM of apo-rCRALBP (100 mM Tris pH 8.0, 7.7 μg of RPE protein, 0.2 % BSA 100 μM of DPPC, 1 mM of DTT 30 μM apo-rCRALBP and 0.2 μM all-*trans*-retinol [11-12-$^3H_2$]) at 37^{0}C for 30 minutes. At the end of this incubation period the 200 μL reaction mixture was quenched by the addition of 750 μL ice cold methanol after which 100 μL of 1M sodium chloride solution was added, and 500 μl hexane (containing butylated hydroxy toluene at 1 mg/mL) was added to effect extraction of the retinoids. The retinoids were analyzed as previously described (27). The amount of 11-*cis*-retinol formed was used as a measurement of IMH activity. All experiments were performed in triplicate and the average values of these measurements were used for analysis.

[0572] Fig. 4 A, B, C shows data for the specific binding of all-*trans*-retinoic acid to purified RPE65. As shown in Fig. 4A, the binding of all-*trans*-retinoic acid to RPE65 led to an exponential decay in protein fluorescence. This decay follows a saturable binding isotherm (Fig. 4B), and yields an average K_D for binding of approximately 109 nM (SD = 10 nM, N = 4) (Fig. 4C). Similar data are shown in Fig. 5 A, B, C for 13-*cis*-retinoic acid, yielding an average K_D for binding of approximately 195 nM (SD = 20 nM, N = 4) (Fig. 5C). These experiments show that both retinoic acids specifically bind to RPE65 with high affinities. By way of comparison, under the same binding conditions, all-*trans*-retinyl palmitate binds to RPE65 with a K_D=47 nM (data not shown). To further assess specificity of binding, the binding interactions of an additional retinoid, N-(4-hydroxyphenyl)retinamide (Fenretinide):



with RPE65 was studied. The binding of N-(4-hydroxyphenyl)retinamide to RPE65 is expected to be considerably weaker than for the retinoic acids, because analogs in the retinamide series only weakly induce night blindness. In fact, N-(4-hydroxyphenyl)retinamide binds rather weakly to RPE65. Data shown in Fig. 6 A, B, C, yield an average K_D for binding of approximately 3547 nM (SD = 280 nM, N = 4) (Fig. 6C). Thus the observed weak binding for N-(4-hydroxyphenyl)retinamide is what is predicted for the hypothesis that RPE65 is the night blindness target.

[0573] B. Retinoic acid displaces all-*trans*-retinyl palmitate from RPE65.

[0574] Competitive binding of retinoic acid (all-*trans* and 13-*cis*) and all-*trans*-retinyl palmitate to RPE65: Buffer exchange experiments were performed to investigate the abilities of the retinoic acids (all-*trans* and 13-*cis*) to displace all-*trans*-retinyl palmitate binding from RPE65. To RPE65 (0.5 μM) (PBS, 1% CHAPS, pH 7.4), was added 6 μM of retinoic acid (all-*trans* and 13-*cis*) and incubated at 4^0C for 30 min. A control sample of RPE65 was incubated minus retinoic acids at 4^0C for 30 min. At the end of this incubation, the samples were incubated for 30 min with ^{3}H-all-*trans*-retinyl palmitate (0.65 μM, 20.31 Ci/mmol). At the end of this incubation period the buffer (PBS-1% CHAPS) was exchanged 10^4 fold with a Centricon 30K MWCO filter. The sample retained and the buffer flow through were counted on a liquid scintillation counter, to measure the amount of ^{3}H-all-*trans*-retinyl palmitate retained.

[0575] The direct binding studies reported above for the retinoic acids are not determinative as to whether these molecules are competitive with the binding of all-*trans*-retinyl esters, the physiologically relevant ligands of RPE65. This can readily be shown by pre-binding ^{3}H-all-*trans*-retinyl palmitate to RPE65 and showing that all-*trans*-retinoic acid competes with the binding (Fig. 7). This experiment was performed by first incubating RPE65 with all-*trans*-retinoic acid or 13-*cis*-retinoic acid and (-) retinoic acid (control), following which the protein was incubated for 30 min with ^{3}H-all-*trans*-retinyl palmitate. Excess retinoids were removed by buffer exchange and the flow through and retained solutions were counted using a liquid scintillation counter. The data show that the retinoic acids and all-*trans*-retinyl esters apparently compete for the same binding-site on RPE65.

[0576] C. Retinoic acids inhibit RPE65 function

[0577] RPE65 is the chaperone for all-*trans*-retinyl esters and, as such, is essential for the mobilization of these hydrophobic molecules for isomerization. In the current studies, a bovine retinal pigment epithelial membrane system is used to process added all-*trans*-retinol (vitamin A) to form 11-*cis*-retinol. Since RPE65 is essential for the biosynthesis of 11-*cis*-retinol (4,8,11), inhibitors of it could block this synthesis. In the experiments shown in Figure 7 the off-rate for the binding of all-*trans*-retinyl esters to RPE65 is sufficiently slow to allow the complex to survive centrifugation on Centricon spin columns. The same is true for all-*trans*-retinoic acid (data not shown). This suggests that the order of incubation of inhibitors of RPE65 would be expected to be important to reveal effective inhibition. Pre-incubation of these membranes with vitamin A rapidly produces all-*trans*-retinyl esters through rapid esterification mediated by LRAT. The synthesized all-*trans*-retinyl esters are tightly bound to RPE65, and then processed into 11-*cis*-retinol by IMH. This system is not susceptible to inhibition by all-*trans* or 13-*cis*-retinoic acids incubated at 60 μM (data not shown). This is the expected result because the retinoic acids are known not to directly inhibit IMH. However, pre-incubation with the retinoic acids produced a markedly different result, as shown in Figure 8. In this case, substantial inhibition of 11-*cis*-retinol formation occurs in the presence of the retinoic acids because they have access to RPE65. Interestingly, the inhibition observed with N-(4-hydroxyphenyl)retinamide proved to be substantially weaker (Figure 8). This is the expected result, given its relatively weak affinity for RPE65.

[0578] D. The Stereospecific Binding of Vitamin A by sRPE65

[0579] As mentioned above, membrane associated RPE65 (mRPE65) stereospecifically binds all-*trans*-retinyl palmitate. In this example, the binding of retinoids to sRPE65 is measured by the fluorescence methodology already described (Gollapalli, D.R., Maiti, P., Rando, R.R. (2003) RPE65 operates in the vertebrate visual cycle by stereospecifically binding all-trans-retinyl esters. Biochemistry *42*, 11824-30.). The excitation wavelength was at 280 nm and the emission was observed through 0.5 cm layer of solution. The titration solution consisted of 0.37 μM of sRPE65 in 100 mM phosphate buffered saline (150 mM) pH 7.4 and 1% CHAPS. In Figure 9 A, B are shown data for the binding of all-*trans*-retinol (tROL) and all-*trans*-retinyl palmitate to purified sRPE65. Figure 9A1 shows the emission spectra of sRPE65 with increasing concentrations of tROL. Figure 9A2 shows the linear squarefit plots for the titration of sRPE65 vs. tROL. As shown in Figure 9A, the

binding of all-*trans*-retinol to sRPE65 led to an exponential decay in protein fluorescence which followed a saturable binding isotherm and yielded an average K_D (Figure 9D) for binding of approximately 65 nM (Figure 9A2). In Figure 9B are shown data for the binding of all-*trans*-retinyl palmitate (tRP) to sRPE65 with a similar exponential decay in protein fluorescence. Figure 9B1 shows the emission spectra of sRPE65 with increasing concentrations of tRP. Figure 9B2 shows the linear squarefit plots for the titration of sRPE65 vs. tRP. This decay followed a saturable binding isotherm, and yielded an average K_D (Figure 9D) for binding of approximately 1.2 μM (Figure 9B2). The Binding Constants of tROL and rRP with mRPE65 and sRPE65 with 1% CHAPS in 100 mM phosphate buffer with 150 mM sodium chloridebinding data are compiled in Figure 9D.

[0580] E. sRPE65 as a Vitamin A chaperone in the formation of all-*trans*-retinyl esters.

[0581] Vitamin A bound to sRPE65 is shown to be metabolically active by demonstrating its ability to be processed by LRAT, an enzyme which represents the only known metabolic route for vitamin A processing in the RPE. As shown in Figure 9C, vitamin A bound to sRPE65 is an excellent substrate for truncated LRAT (tLRAT), a readily expressed form of LRAT, which is mechanistically indistinguishable from LRAT. These studies demonstrate that sRPE65 can indeed direct vitamin A to LRAT, and thus the binding of vitamin A to sRPE65 may have functional significance. In the absence of sRPE65 very little synthesis of all-*trans*-retinyl palmitate occurs (Figure 9C). As reported in Figure 9C, tRP was produced in the presence of (1) sRPE65 (0.04 μM), dodecyl maltoside (0.1%) and DPPC (200 μM), but not (2) dodecyl maltoside (0.1%) and DPPC (200 μM) or (3) sRPE65 (0.04 μM) alone. All reaction mixtures contain 100 mM Tris pH 8.4, 1 mM dithiothreitol, 1mM EDTA, 5 μM tLRAT and 0.2 μM tROL.

[0582] F. Palmitoylation of sRPE65

[0583] The biochemical relationship between mRPE65 and sRPE65 was studied with respect to their hydrophobic post-translational modification states. S-palmitoylation seemed the most likely possibility given that the process is reversible. This can be directly tested in a standard way by growing insect cells (*sf*21) transfected with rHRPE65 baculovirus (Ma, J., Zhang, J., Othersen, K.L., Moiseyev, G., Ablonczy, Z., Redmond, T.M., Chen, Y., Crouch, R.K. (2001) Expression, purification, and MALDI analysis of RPE65. Invest Ophthalmol Vis Sci. *42*,1429-35) in $^3H_2$-palmitic acid and determining whether the expressed mRPE65 is labeled. Figure 10A shows the *in vivo* palmitoylation of rHmRPE65, expressed in *sf*21 cells in the presence of $^3H_2$ palmitic acid and separately in

the presence of unlabeled palmitic acid. L1-4 shows the Coomassie stained gel, L5-6 shows the autoradiogram of L1-4 and L8 shows the Western blot of rHmRPE65. In panel A, (L1) shows the ^{14}C molecular weight markers; (L2) shows the control with purified rHmRPE65 expressed in *sf*21 cells grown in the presence of unlabeled palmitic acid (0.09 μM); (L3) shows where purified rHmRPE65 expressed in *sf*21 cells in the presence of $^{3}H_2$ palmitic acid (0.09 μM-0.5mCi/mL) and treated for 16hrs with 0.5 M Tris pH 8.0; (L4) shows purified rHmRPE65 expressed in *sf*21 cells in the presence of $^{3}H_2$ palmitic acid (0.09 μM-0.5mCi/mL) and then treated for 16h with 0.5 M hydroxyl amine pH 8.0; (L5, L6 and L7) show the autoradiograms of L2, L3 and L4. L7 shows the Western blot for purified rHmRPE65 detected with anti-RPE65 primary antibody (1:4000-1hrs room temperature).

[0584] As shown in Figure 10A, purified mRPE65, expressed in insect cells, is indeed labeled by added ^{3}H-palmitic acid. As expected, treatment of the labeled mRPE65 with hydroxylamine, which cleaves thioesters, releases the label. In order to define the sites of *in vivo* modification of mRPE65, mass spectroscopic experiments were performed on purified bovine mRPE65 and sRPE65. These samples were digested with trypsin and subjected to mass spectroscopic analysis (Figures 10 B and C). The results show that mRPE65 is triply palmitoylated at positions C231, C329, and C330. By comparison, the data also show that sRPE65 appears not to be palmitoylated.

[0585] Figures 10B and C show the mass spectrometry analysis of two different peptides from mRPE65 and sRPE65. Trypsin digested RPE65 peptides were analyzed by MALDI-TOF. Peak annotations are as follows: Figure 10B, 1378.9 Da (amino acid sequence 223-234, SEIVVQFPCSDR), 1429.4 Da (1-14, N-Acetyl-SSQVEHPAGGYKK), 1477.4 Da (34-44, IPLWLTGSLLR), 1483.0 Da (114-124, NIFSRFFSYFR), 1616.6 Da (223–234, SEIVVQFPC*SDR), 1700.1 Da (83–96, FIRTDAYVRAMTEK), 1701.7 (367-381, RYVLPLNID), 1718.7 (83-96, FIRTDAYVRAM#TEK). Figure 10C, 2770.3 (333-354, GFEFVYNYSYLANLRENWEEVK), 3321.6 (306-332, TSPFNLFHHINTYEDHEFLIVDLCCWK), 3797.8 (306-332, TSPFNLFHHINTYEDHEFLIVDLC*C*WK). C* denotes palmitoylated cysteine and M# for oxidized methionine.

[0586] G. The interconversion of mRPE65 and sRPE65 by LRAT

[0587] Since mRPE65 and sRPE65 exhibit complementary retinoid binding specificities, it is essential to understand how these two molecules are inter-converted. Most interestingly, LRAT is able to utilize mRPE65 as a palmitoyl donor (Figure 11A) and transfers this

moiety to vitamin A to generate all-*trans*-retinyl palmitate (Figures 11 B, C, D). Figure 11B shows the mRPE65 alone (-■-) and DPPC alone (-●-) dependent esterification of all-*trans*-retinol. In these experiments, pure mRPE65 and vitamin A are incubated with LRAT and the resultant all-*trans*-retinyl palmitate is isolated by HPLC. Mass spectroscopic analysis of the isolated all-*trans*-retinyl palmitate show it to be authentic and hence a palmitoyl moiety was transferred from mRPE65 to vitamin A. These data also reveal that mRPE65 is a much more efficient palmitoyl donor than dipalamitoyl phosphatidylcholine (DPPC), the standard acyl donor in the LRAT reaction. Under the same conditions, no observable turnover of DPPC is measured (Figure 11B). The kinetic plot shown in Figure 11B reveals sigmoidal kinetics with a calculated K_M (K_{app}) of 0.03 μM for mRPE65 (compared to a value of 1.4 μM for DPPC). The Hill plot (Figure 11C) yields a value of 2.54 for N, suggesting that more than one molecule of mRPE65 is involved in the transfer of the palmitoyl group. The observed sigmoidal kinetics suggests a regulatory role for this process, allowing it to respond to slight changes in mRPE65 concentrations.

[0588] Reversibility in the reaction is readily established. In these experiments (Figure 11D) excess all-*trans*-retinol is incubated with mRPE65 and tLRAT until no further all-*trans*-retinyl palmitate is generated. This is followed by treatment with ^{3}H-*trans*-retinol. The subsequent rise of the specific activities of all-*trans*-retinyl palmitate and the fall in specific activities of all-*trans*-retinol at constant all-*trans*-retinyl palmitate levels reveals the equilibration of substrates (Figure 11D). Figure 11D shows the change in specific activities (left y-axis) of tRP (-■-) and tROL (-●-) as a function of time. The total retinyl ester (-▲-) formed (right y-axis) shows the saturation of the ester synthesizing reaction. Each reaction contains 100 mM Tris pH 8.4, 0.06 μM mRPE65, 5 μM tLRAT, 1 mM dithiothreitol, 1 mM EDTA and 10 μM tROL.

[0589] The depalmitoylated RPE65 formed when mRPE65 is treated with tLRAT and vitamin A shows retinoid binding behavior similar to sRPE65. mRPE65 was incubated with excess vitamin A and tLRAT. After the removal of the retinoids and tLRAT, the sRPE65 was then studied with respect to its ability to bind vitamin A and all-*trans*-retinyl palmitate . As shown in Figure 2D, the treatment of mRPE65 with LRAT and vitamin A, converts mRPE65 to a functional binding form of RPE65 indistinguishable from that of isolated sRPE65.

[0590] H. 11-*cis*-retinol is Palmitoylated by mRPE65 and LRAT

[0591] 11-*cis*-retinol, the direct product of IMH action, is also esterified (Figure 12A). As Figure 12B shows, 11-*cis*-retinol is actually a superior substrate during the tLRAT/mRPE65 mediated esterification (palmitoylation) of the retinoids compared to vitamin A. (1) 11-*cis*-retinol (2 μM) and mRPE65 (0.02 μM). (2) all-*trans*-retinol (2 μM) and mRPE65 (0.02 μM). (3) 11-*cis*-retinol (2 μM) and DPPC/BSA (250 μM/0.4%). (4) all-*trans*-retinol (2 μM) and DPPC/BSA (250 μM/0.4%). All reaction mixtures contain 100 mM Tris pH 8.4, 1 mM dithiothreitol, 1 mM EDTA and 5 μM tLRAT.

[0592] The palmitoylation of 11-*cis*-retinol by mRPE65 provides a natural mechanism through which mRPE65 is turned over and control is exerted during the operation of the visual cycle because 11-*cis*-retinol drives mRPE65 to sRPE65, effectively shutting down the pathway to chromophore biosynthesis. This is directly demonstrated in Figure 12C in which RPE membranes are treated with 10 μM 11-*cis*-retinol to drive the mRPE65 to sRPE65 transition. This figure shows the time dependent generation of [11-12-$^3H_2$] 11-*cis*-retinol in the presence of 11-*cis*-retinol mediated depalmitoylation (-●-) and in the absence of 11-*cis*-retinol mediated depalmitoylation (-■-). The inset shows the full time interval. Irradiation of the sample with ultraviolet light destroys the 11-*cis*-retinoids. Control and treated samples are then incubated with vitamin A, and the rates of 11-*cis*-retinol, the product of IMH, are measured. The sample pretreated with 11-*cis*-retinol shows a distinct lag period before product synthesis occurs.

[0593] I. Exemplary RPE65 antagonists

[0594] The constants of affinity (K_ds) of several compounds for mouse RPE65 were determined. It was found that the compounds described above as 4a, 4b and 4c have a *Kd* of 47 nM, 235 nM and 1300 nM, respectively. Thus, the potency of binding is a function of the ester chain length, i.e., the longer the chain length, the stronger the affinity for RPE65 is and the stronger the antagonist is.

[0595] Compounds listed above as 4d, 4e and 4f are also potent RPE65 antagonists, having a K_d of 21 nM, 40 nM and 64 nM, respectively.

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[0648] Example 2: Effect on visual cycle of short-circuiting drugs in vivo

[0649] Mice were injected intraperitoneally (i.p.) with 50 mg/kg of the compounds listed prepared in 25 microliters DMSO. Positive control mice were injected with 13–*cis*-retinoic acid (ACCUTANE®) 50 mg/kg in 25 microliters DMSO. Negative control mice were injected with 25 microliters DMSO.

[0650] At predetermined times after administration, mice were exposed to sufficient light to cause complete bleaching of the visual cycle. Electroretinograms (ERG) were then performed in bright light or dim light, and the b-wave amplitude measured. The b-wave amplitude is assumed to be proportional to rhodopsin regeneration and thereby correlate with the functioning of the visual cycle (i.e., the higher the b-wave amplitude, the greater the functioning of the visual cycle).

[0651] A. 4-butyl-aniline and ethyl 3-aminobenzoate

[0652] 4-butyl-aniline and ethyl 3-aminobenzoate, were prepared as solutions in DMSO. 7 months old wild type (wt; C57BL/6J X 129/SV; Rpe65 Leu450Leu) mice were injected i.p. with 25 microliters (50 mg/kg) of each compound. Animals injected with ACCUTANE (13-*cis*-retinoic acid, 25 microliters, 50mg/kg) and DMSO (labeled as wt; 25 microliters) were used as positive and negative controls, respectively. Two mice were injected in each group. ERG measurements were performed.

[0653] FIG. 14A shows effects of the compounds 1 hour after injection. The wild-type negative control showed a recovery to 50% of baseline b-wave amplitude (considered

complete recovery), while the positive control and test compounds showed greater impairment of the visual cycle.

[0654] FIG. 14B shows effects of the compounds 1 week (7 days) after injection. The test compounds had a sustained effect, while the positive control returned to complete recovery.

[0655] FIG. 14C shows effects 2 weeks (14) days after injection. The test compounds still had effect on the visual cycle.

[0656] B. Ethyl-(3-N-methl)amino benzoate, N-Methyl-4-butyl aniline

[0657] FIGS. 15A-B, 16A-B, and 17A-B show, respectively, three experiments with these compounds in dim (A) and bright (B) light.

[0658] C. Ethyl-(2-N-methl)amino benzoate, N-Methyl-4-butyl aniline

[0659] FIGS. 18A-B show experiments with these compounds in dim (A) and bright (B) light.

[0660] Example 3: Effect on visual cycle of enzyme inhibitors and RPE65 antagonists in vivo

[0661] The experiments described in Example 2 were repeated additional compounds.

[0662] A. Retinyl palmityl ketone and retinyl decyl ketone

[0663] FIG. 19 shows an experiment with these compounds.

[0664] B. All-trans-retinyl palmityl ketone, all-trans-retinyl palmityl ether

[0665] FIGS. 20A-B and 21A-B show, respectively, two experiments with these compounds in dim (A) and bright (B) light.

[0666] C. Octyl farnestimide, palmityl farnestimide

[0667] FIG. 22A shows the results of an experiment in dim light using these compounds shortly after administration. FIG. 22B shows the results one week after administration.

[0668] E. Farnsyl octyl ketone, farnesyl decyl ketone

[0669] FIGS. 23A-C show experiments performed in dim light using these compounds. The data shown in FIG. 23B was obtained 3 days after administration. The data in FIG. 23C was obtained 8 days after administration.

[0670] F. Farnesyl palmityl ketone, farnesyl decyl ketone

[0671] FIG. 24 shows the results of an experiment performed in dim light 1 hour after these compounds were injected.

[0672] Example 4: A_2E formation in the presence of aromatic amine.

[0673] 100 mg (355 μmoles) of all-*trans*-retinal was dissolved in 3 mL followed by the addition of 9.5 μL of glacial acetic acid 9.5 μL of ethanolamine (155 μmoles). This

solution was aliquoted into 300 μL fractions. 3-aminoethylbenzoate (15.5 μmoles) was added to the samples at 0, 2, 3.75, 14, 16.25, 18.0833 19.917 23.75 and 48hrs. A control sample did not contain aromatic amine. The solutions were shaken at room temperature for 48hrs. The solutions were then kept at –80°C and 15 μL was diluted to 250 μL (methanol). The solutions (15 μL) were then analyzed on a reverse phase (C18-5 μm-4.6mmx150mm) column on a linear gradient 85%-96% methanol/water and UV detector at 430 nm to determine the amount of A_2E formation. The percent A_2E formation was compared to A_2E formation at 48 h in the absence of aromatic amine.

[0674] Table 1: A_2E formation

Time (Hrs)	% formation (48 Hrs=100)	Area under the Curve
0	3.98	422.5
2	8.67	920
3.75	7.71	818.5
14	19.73	2094
16.25	19.25	2043
18.0833	23.70	2515
19.91667	27.65	2934
23.75	22.44	2382
48	100.00	10613

[0675] The data in Table 1 is presented in Fig. 25 and shows that aromatic amines can not form A_2E in the standard *in vitro* reaction; furthermore, they prevent A_2E from forming.

[0676] Example 5: In vivo A_2E formation

[0677] Effects on accumulation of A_2E in *abcr* knockout mice by farnesyl decyl ketone and N-palmityl farnesimide were investigated. Mice were injected with 50 mg/kg drug in 25 microliters of DMSO or with only DMSO for the control group once or twice a week. After 2 to 2 1/2 months, the mice were sacrificed, and A_2E and iso-A_2E were harvested and quantitated from 4 eyes for each drug or control. Results are shown in Table 2.

[0678] Table 2: A_2E Accumulation in treated or untreated *abcr* mice

	(pmoles/eye)		
	A2E	iso-A2E	total
no drug	11.01	2.57	13.58
Farnesyl decyl ketone	2.00	0.66	2.66
N-Palmityl farnesimide	5.38	1.70	7.09

[0679] These data show that farnesyl decyl ketone reduced A_2E accumulation by over 80%, iso-A_2E by about 74%, total by about 80%. N-palmityl farnesimide reduced A_2E accumulation by over 50%, and iso-A_2E by about 33%, total by about 47%.

[0680] The examples should not be construed as limiting in any way. The contents of all cited references (including literature references, issued patents, published patent applications as cited throughout this application) are hereby expressly incorporated by reference.

[0681] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments described herein.